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TRANSPLANTATION OF TISSUES IN HYBRIDS OF INBRED FAMILIES OF GUINEA PIGS AND THE INDIVIDUALITY DIFFERENTIAL[1]

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IN a former paper Loeb and Wright[2] have analyzed individuality differentials by means of transplantations within inbred families of guinea pigs. They also carried out transplantations between brothers and sisters within inbred families and compared the results thus obtained with those after transplantation from one inbred family to another and after ordinary homoio, syngenesio and autotransplantation. In addition they studied the effect of transplanting from a hybrid between two inbred families to one of the parent families or *vice versa* from one of the parent families to a hybrid. The results obtained in the latter experiments made it possible to define the conditions under which reciprocal transplantations may yield different results, and furthermore indicated the cause of the reactions of the host against the transplant.

According to the theory of the individuality differential[3] we should expect that if we carry out transplanta-

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[2] Leo Loeb and Sewall Wright, *Amer. J. Path.*, 1927, 3, 251.

[3] Leo Loeb, AM. NATURALIST, 1920, 54, pp. 45 and 55; *Physiol. Reviews*, 1930, 10, 547.

tions from hybrids between two inbred strains to other hybrids between the same strains or to hybrids in which only one of the component strains is the same while the other differs, the results are less favorable than if we transplant tissues within the same strain, because in the former case the chances that the genetic constitution of the donor tissue differs from that of the host tissues is greater than in the latter cases. During the last two years we undertook a series of such transplantations with a view of testing this supposition. While the number of animals which was at our disposal for this purpose was unfortunately not very large, we believe that, notwithstanding this deficiency, the results obtained are definite in several respects.

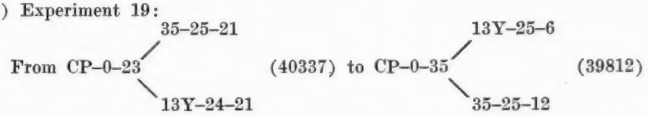
In the paper of Loeb and Wright there were recorded only two experiments in which tissues were exchanged between hybrids composed of the same two parent strains. In one of those two experiments a hybrid between strains 2 and 35 was used. Examination took place after 31 days and the result was 5, corresponding to syngenesiotransplantation. In the second experiment a hybrid between strains 35 and 32 was used, examination took place after 35 days and the result was 6, corresponding to autotransplantation.

In our present series two kinds of hybrids were at our disposal: (1) Hybrids between families 35 and 13, designated as CP; and (2) hybrids between families 13 and 2, designated as CY. In CY–0, a line of family 13, rich in individuals with otocephalic defects, was used and in CP–0 the same line of family 13 was likewise used in many cases. CP–0 and CY–0 represent the F_1 hybrids between these families. CP–1 and CY–1 designate the first generation of brother-sister matings from experiment CP–0 and CY–0, respectively, while CP–2 and CY–2 represent the corresponding second generations of brother-sister matings.

We shall give a list of the experiments of this kind carried out and of the results obtained. While as a rule we

shall state the results briefly, in a few cases we shall describe them in somewhat more detail.

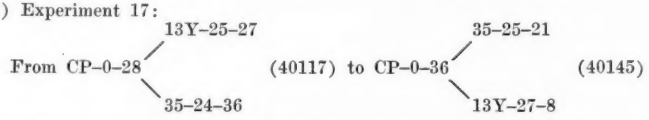
(1) Experiment 19:



Examination after 20 days. Results approach but do not quite equal those of autotransplantation. The thyroid transplant resembles, as far as its structure is concerned, an autotransplant, except that in some places there is near the border of the inner ring of the transplant a certain amount of dense fibrous tissue. The ring of acini is well preserved; the acini and the colloid they contain are normal and usually the acini are close together. However, the number of lymphocytes is slightly increased; they fill loosely some of the small lymph vessels. The fat tissue around the thyroid is, on the whole, well preserved, but there is in places a little too much connective tissue growing into it and there is also here and there a small increase in lymphocytes. We find, furthermore, a new formation of epithelioid cells. While the parathyroid is on the whole well preserved, one part of this structure has been almost entirely replaced by masses of lymphocytes, still enclosing remnants of parathyroid tissue and a number of lymphocytes are seen infiltrating the neighboring parathyroid. The cartilage is well preserved although some parts of it are necrotic probably owing to the injury received during the process of transplantation; there are in places small collections of lymphocytes in the connective tissue surrounding it. The fat tissue around the cartilage is also well preserved and contains few lymphocytes. The transplanted muscle fibers show normal cross striation and nuclear chains as an indication of regenerative processes which have taken place. The surrounding connective tissue shows an increase in the number of lymphocytes. The transplanted bone is in part surrounded by osteoblastic tissue and it is possible that some light staining areas of bone are

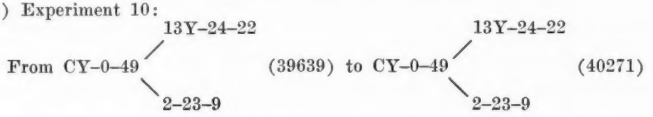
newly formed. The bone marrow is replaced by large capillaries and some loose connective tissue. There are probably some bone marrow cells (myelocytes) left.

(2) Experiment 17:



Examination after 41 days. The result approaches that of homoiotransplantation. Instead of thyroid transplant, only fibrous tissue is found. The cartilage transplant is mostly preserved, including the columns of cartilage cells near the bone; however, a thin zone of necrotic cartilage is in process of calcification. The cartilage is surrounded by fibrous tissue. Where the cartilage is necrotic, connective tissue penetrates into it. Also a mass of lymphocytes pushes its way into the center of the cartilage, where it is apparently undergoing calcification; furthermore, masses of lymphocytes enter the cartilage-bone border. In addition there is some lymphocytic infiltration in the connective tissue around the cartilage. Here the connective tissue is finer in structure than is found in typical cases of homoiotransplantation. In this specimen the reaction of the host tissue against the transplant seems to be less violent. The bone is necrotic and the bone marrow is replaced by loose fibrillar connective tissue. Around the bone there is fibrous tissue with lymphocytic infiltration of medium intensity. Near the cartilage-bone border the fat tissue contains epithelioid and giant cells.

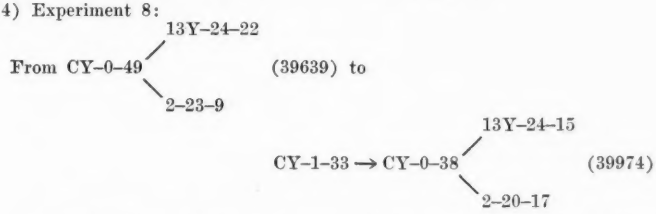
(3) Experiment 10:



Examination after 21 days. Host and donor have in this case the same genetic constitution, although they are not brothers. In accordance with expectation the result corresponds to that of autotransplantation. Thyroid,

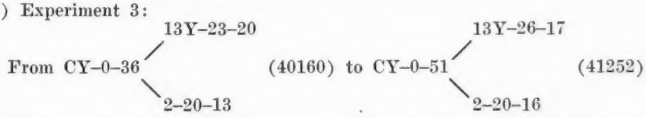
parathyroid, cartilage, bone, muscle and fat tissue behave as they do in case of autotransplantation. The transplant as a whole is very well preserved, and also the bone marrow is at least partly preserved, while another part of it is necrotic. Near the cut end it is replaced by loose connective tissue which has penetrated into it from the outside. The number of lymphocytes does not exceed that seen in autotransplants.

(4) Experiment 8:



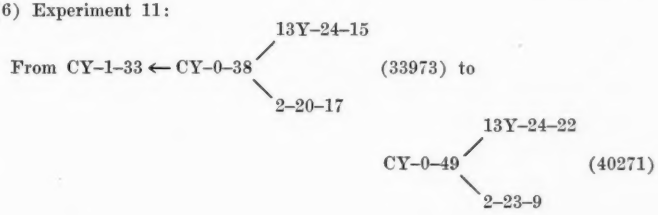
Examination after 21 days. The constitution of host and donor are not identical, but similar. Results correspond about to autotransplantation.

(5) Experiment 3:



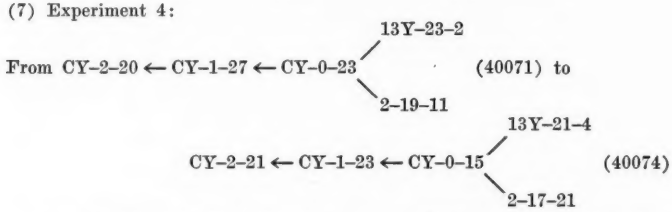
Examination after 25 days. Constitution as far as family 2 is concerned is very similar; there is, however, a slight difference in regard to family 13. In this case thyroid and parathyroid behave like autotransplants; the necrosis of great parts of the transplanted cartilage may be due to accidental conditions. We find in the bone marrow, which is partly preserved, some newly formed osteoid tissue as the result of the activity of osteoblastic tissue.

(6) Experiment 11:



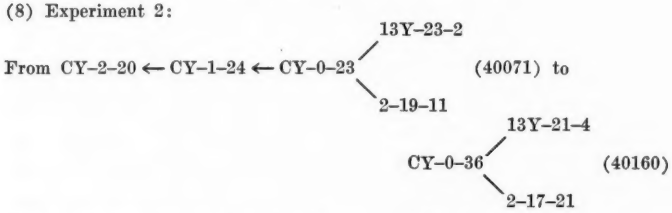
Examination after 21 days. Constitution of family 13 in these hybrids is very similar, but there is some difference in the constitution of family 2. As far as preservation and structure of thyroid, cartilage and bone marrow are concerned, they correspond about to autotransplantation; however, there is some increased lymphocytic infiltration in thyroid and around cartilage and some increase in fibrous tissue in various places including the fat tissue. The results correspond to those seen in favorable cases of syngenesiotransplantation.

(7) Experiment 4:



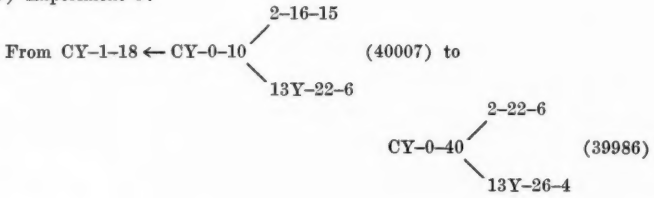
Examination after 25 days. In this case constitution of family 13, as well as of family 2, differs somewhat in both hybrids. Homoioreaction.

(8) Experiment 2:



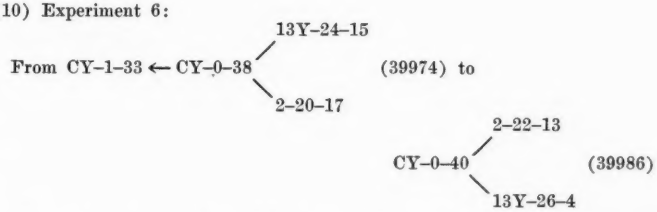
Examination after 25 days. The hybrids differ in constitution of family 13 as well as of family 2. On the whole, homoioreaction.

(9) Experiment 5:



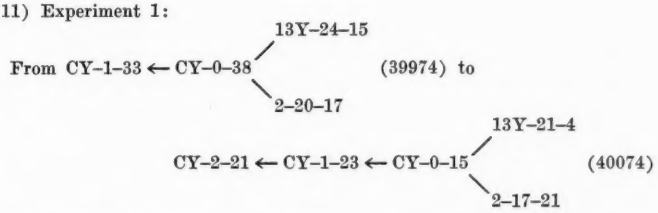
Examination after 20 days. Homoioreaction. The hybrids differ in the constitution of family 13 as well as of family 2.

(10) Experiment 6:



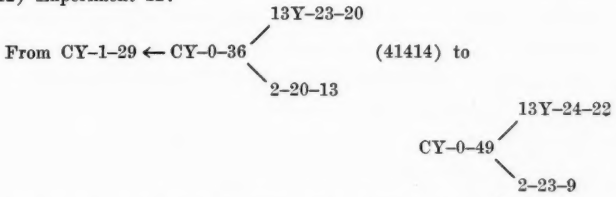
Examination after 20 days. Homoioreaction. Both hybrids differ in constitution of family 13 as well as of family 2.

(11) Experiment 1:



Examination after 20 days. Homoioreaction. Family 13 as well as family 2, entering these hybrids, differ somewhat in constitution.

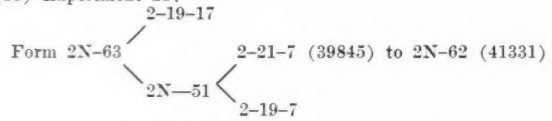
(12) Experiment 12:



In this case the uterus had been transplanted. Examination after 10 days. Surface epithelium, glands as well as muscle tissue are fairly well preserved; but parts of transplant are necrotic. There are some small collections of lymphocytes. The reaction was unlike that found in autotransplantation.

In addition, we carried out two experiments in which instead of hybrids between 2 families, hybrids between different branches within the same family were used for transplantation. We have therefore in these cases not to deal with as closely inbred individuals as in the transplantations within the same strain used formerly by Loeb and Wright, where there had been consistent brother and sister mating. The animals designated "2N" were selected from family 2 for colored nose spots.

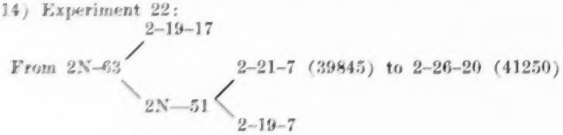
(13) Experiment 21:



2N–62 has a very complicated pedigree. It comes through 9 generations of selection on the sire's side for pigmented nose spots (non-brother-sister matings) to the strict brother and sister matings in inbred family 2. The sire's sire and dam, however, are brother and sister from the selection experiment. On the dam's side there are 3 generations of non-brother-sister matings till we reach the continued brother-sister matings in family 2.

Examination after 25 days. Thyroid is only partly preserved. In the majority of the acini no colloid is left. While there is only a very slight lymphocytic reaction, there is an increased amount of connective tissue around some acini. Cartilage is on the whole well preserved. In the fat tissue there is in places a slight connective tissue ingrowth and a slight epithelioid reaction. There is a very moderate lymphocytic infiltration around the cartilage and also in the fat tissue there are some lymphocytes. Bone marrow is not preserved. We have to deal here with a syngenesio, approximately intermediate between an autogenous and a homogenous reaction.

(14) Experiment 22:



Examination after 31 days. In this case the host had been inbred through 26 generations of brother and sister matings. Thyroid, cartilage, bone and bone marrow are relatively well preserved, but there is a slight increase in lymphocytes and a very moderate increase in connective tissue. We have therefore to deal with a less severe syngenesio reaction than that obtained in the preceding Experiment 21.

Discussion

In this series we have to deal with 12 experiments in which tissues were exchanged between hybrids in the constitution of which two different inbred families entered: but in each experiment the donor and host were offspring from the same parent strains. In two additional experiments host and donor belonged to the same family, but either host and donor were not the result of strict brother and sister matings, but were for several generations hybrids between individuals other than brother and sister of family 2, or the donor was such a hybrid, while the host was closely inbred.

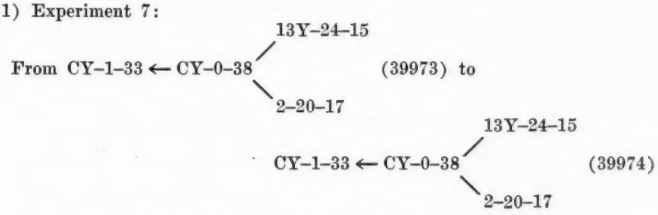
The first 12 experiments can be divided into two classes: a first one (a) consisting of five experiments in which the results approached or even reached the conditions characteristic of autotransplantation, including one case with a result corresponding to a favorable syngenesiotransplantation, and a second one (b) consisting of six experiments in which the results approached or reached those characteristic of homoiotransplantation. There was one experiment (12), in which, although the examination took place too early to allow of a definite grading of the result, we can be certain that it did not correspond to the condition characteristic of autotransplantation. In class (a) each of the two families, which entered into the constitution of donor and host, belonged to the same generation of brother and sister mating

within that particular family, while in (b) this held good only in the case of one of the families entering the constitution of this hybrid, while in the case of the other family the generations differed. This sharp division between the behavior of these two kinds of hybrids may be a coincidence, if we consider the results as a whole, but in individual cases the more favorable outcome of the transplantation may be due to a closer relationship existing between the donor and the host in class (a) than in class (b).

In the last two experiments (13 and 14), the results were inferior to the average results obtained by Loeb and Wright in transplantations within closely inbred families. Hybridization within the same family may be expected to lead to a slightly less homogeneous genetic condition in donor and in host than in case donor and host have been propagated strictly by brother and sister matings within the same family. Greater still are the chances for a difference in constitution in the first 12 experiments. Where two different families enter the constitution of each family, there is a greater probability that the genetic constitution of donor and host are dissimilar than when both father and mother belonged to the same inbred family. Correspondingly the results obtained in these transplantations are much less favorable than the results obtained by Loeb and Wright in transplantations within the same inbred family. In the latter experiments the results varied between those characteristic of auto and syngenesiotransplantation. In the new experiments in about one half of the cases results were obtained approaching those found in homoiotransplantation. While in the earlier transplantations the average results were definitely superior to those noted in brother to brother transplantations in ordinary, non-inbred stock of guinea pigs, in this series they are very similar or only very little superior to those obtained in brother to brother matings in non-inbred stock.

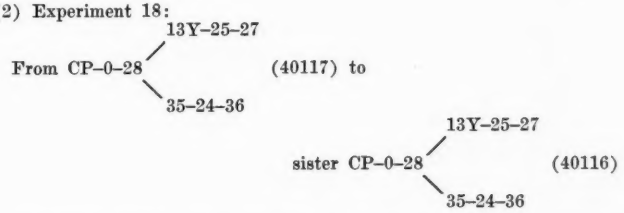
Transplantation from Brother to Brother (or Sister), When Father and Mother Belong to Different Inbred Families

(1) Experiment 7:



Examination after 21 days. In this case transplanted cartilage, fat and muscle tissue approximated in their condition autotransplants; they were on the whole well preserved. Only very few lymphocytes were seen and there was hardly any increase in connective tissue. Cartilage and muscle tissue showed areas of regeneration. The regeneration of cartilage as usual was observed either in places where the tissue had been wounded or where preserved perichondrium surrounded necrotic cartilage.

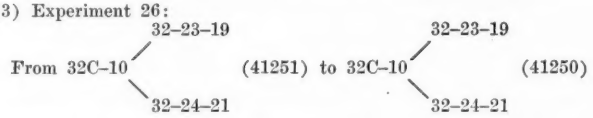
(2) Experiment 18:



Examination after 41 days. Thyroid and surrounding fat tissue on the whole showed the character of autotransplants; but there were small collections of lymphocytes in various places. The same condition was found in the case of cartilage, bone, bone marrow and muscle transplants. There was in places a slight increase in connective tissue and there were some small collections of lymphocytes. The transplants approached, therefore, but did not quite reach the condition found in autotransplantation.

In addition, in two cases brother to brother transplantations between hybrids within the same family were made. The guinea pigs represented the F_1 generations of a mating between two guinea pigs both belonging to family 32, the parents not being brother and sister.

(3) Experiment 26:



The animals are brothers. Examination after 25 days. Thyroid, cartilage, fat and muscle tissue behaved like autotransplants.

(4) Experiment 27. The reciprocal transplantation to preceding experiment (41250 to 41251). Examination after 25 days. Tissues behaved like autotransplants.

DISCUSSION

While these experiments are few in number they are in harmony with the previous results of Loeb and Wright. In accordance with our earlier observations we find that the tissues behave like autotransplants, if brother and sister belong to the same inbred family; this holds good even if father and mother were not brother and sister, but belonged to the same inbred strain. However, we may expect that if a very large series of transplantations of this kind were compared with brother and sister transplantations in strictly inbred families, the average result in the former series would not be quite so good as in the latter. With our small number of experiments this result is not manifest. However, if brother and sister represent hybrids between two different inbred families, instead of belonging to the same closely inbred family, the results, although still very good and approaching those of autotransplantation, are distinctly poorer than in the case of brother and sister transplantations within the same closely inbred family.

If we combine our present results with the previous ones of Loeb and Wright, we obtain the following re-

sults: In case of transplantation within the same inbred family (including two F_1 hybrid transplantations within the same inbred family) we obtained in 14 out of 16 transplantations auto reactions, in one case the result approached an auto reaction, and in the last case a syngenesio reaction was obtained. We carried out altogether 10 transplantations in which brothers or sisters were hybrids between two different inbred families. In 6 of these cases the results corresponded to those obtained in autotransplantation, while in 3 cases the result approached but did not reach that of autotransplantation and in one case a syngenesio reaction was found. In accordance with theoretical expectations, we find then that hybridization, in increasing the probability of a difference in the genetic composition of host and donor, makes the result of brother and sister transplantations somewhat less favorable. However, if we compare the results of the latter type of brother and sister transplantations with the ordinary kind of brother and sister transplantations, as carried out by Loeb[4] and by Loeb and Wright,[1] the result in the latter experiments is much inferior, as might be expected if we consider that in ordinary brother to brother (or sister) transplantations the chances that the genetic composition of two brothers is the same are much poorer than in cases of transplantation within the same inbred families where the genetic constitution is more homogeneous.

Homoiotransplantations

In two cases we carried out transplantations between animals which genetically were not related to each other, although in one of these two cases in which a B animal was used as the host, host and donor ultimately were derived from the same stock. The B group, a considerable number of years ago, originated from the same stock as the inbred families, but, in breeding the B group,

[4] Leo Loeb, *J. Med. Research*, 1918, 38, 393; 39, 39, and 1920, 41, 305; *Am. J. Path.*, 1927, 3, 29 and 45.

matings as close as those between second cousins have been avoided in contrast to the close inbreeding practiced in the case of the inbred families.

(1) Experiment 9:

From CY–1–27 ← CY–0–18 → 13Y–21–4 (40040) to B 385 (40100)
CY–0–18 → 2–18–7

Examination after 21 days. Of the thyroid transplant only dense hyaline fibrous tissue with some lymphocytes remains. The fat tissue shows epithelioid reaction. Around the cartilage which is partly preserved and in which some regenerated areas are seen, there is much lymphocytic infiltration, especially around the places of regeneration, and lymphocytes penetrate even into the newly formed, still cellular cartilage. A great part of the fat tissue has been replaced by fibrillar connective tissue. In the remaining fat tissue there is some epithelioid reaction. Bone marrow is replaced by fibrillar connective tissue in which there is some lymphocytic infiltration. The transplanted muscle tissue is necrotic. The result of transplantation corresponds therefore to that to be expected after homoiotransplantation.

(2) Experiment 20:

From CP–23 → 35–25–21 to one of our stock guinea pigs.
CP–23 → 13Y–24–21

Examination after 20 days. In this experiment the results are very similar to those obtained in the preceding experiment. However, there was still a trace of thyroid tissue preserved in this case. The contrast between the results obtained in these homoiotransplantations and in the preceding syngenesiotransplantations is very great and both results are in accordance with expectations.[5]

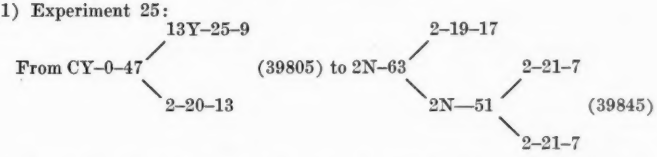
[5] Leo Loeb, *Am. J. Path.*, 1926, Vol. 2, pages 111, 99, 301 and 315.

Transplantation from Hybrid to One of the Component Families and Vice Versa

In former experiments Loeb and Wright found that transplantations from a hybrid between two inbred families to one of the component inbred families gave an average grade of 3.25, signifying a decided reaction on the part of the host against the transplant. In the various experiments the results were as follows: In one case there was an auto reaction; in three cases favorable syngenesio reactions; in eight cases homoio reactions and in twelve cases reactions intermediate between homoio and syngenesio reactions were obtained. It was quite different in transplantations from one of the parent inbred families to a hybrid between itself and a different inbred family. In the latter type of transplantation the average grade was 5.77. In this series the results were as follows: In eight cases the grafts behaved like autotransplants and in the six remaining experiments they behaved like favorable syngenesiotransplants. We can now add to the former experiments four additional ones from our new series.

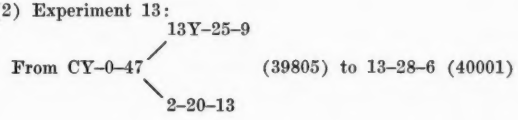
(a) Transplantation from Hybrid to One of the Parent Families

(1) Experiment 25:



Examination after 22 days. A marked homoioreaction took place. No thyroid was preserved; there were strong lymphocytic and connective tissue reactions in the transplants.

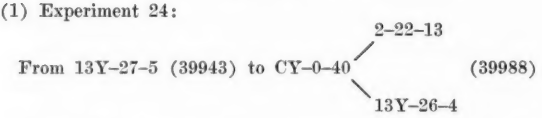
(2) Experiment 13:



In the transplant only very small parts of thyroid tissue were preserved, and there was a distinct lymphocytic reaction. The fat tissue showed epithelioid character; it was partly replaced by fibrous tissue and there was some infiltration with lymphocytes. The cartilage was surrounded by dense fibrous tissue with a moderate or in places even a dense lymphocytic infiltration. While the result obtained in this experiment was somewhat more favorable than in the preceding experiment, it still came within the range of a homoioreaction or at least approached the latter.

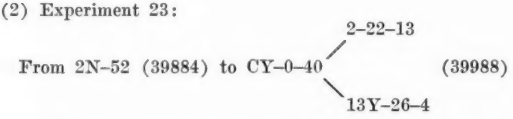
(b) TRANSPLANTATIONS FROM ONE OF THE PARENT FAMILIES TO HYBRID

(1) Experiment 24:



Examination after 31 days. Thyroid as well as cartilage transplant was very well preserved. Both resemble autotransplants except that in one place there is a definite lymphocytic infiltration in the thyroid. While the result in this experiment is in agreement with our former conclusions, the second one behaves in an exceptional way.

(2) Experiment 23:

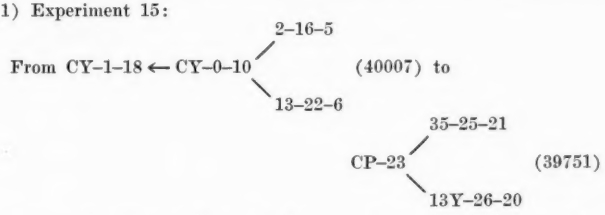


Examination after 31 days. No thyroid was found and the cartilage behaved like a homoiotransplant. Whether in this experiment, which apparently differed in its results from all others, a mistake occurred in the identification of the specimens, or whether the unfavorable outcome is due to the constitution of 2N–52 which represents a very complex 2N hybrid, we must leave undecided.

Transplantation of Tissues in Hybrids, in Which One Parent Family Is the Same in Donor and Host, While the Other Parent Family Differs in Both

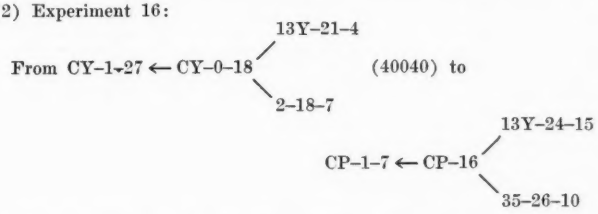
We carried out three transplantations of this kind.

(1) Experiment 15:



Examination after 20 days. A ring of thyroid tissue is preserved; many acini still contain colloid, others are without colloid. In places connective tissue bands separate acini from each other. The connective tissue in the center of the transplant is increased. There is much lymphocytic infiltration; also the parathyroid is partly replaced by collections of lymphocytes. Cartilage is preserved, but surrounded mainly by fibrous tissue. Fat tissue is still partly preserved, but is much infiltrated with lymphocytes. Transplanted muscle tissue is well preserved; but again there is much lymphocytic infiltration in the connective tissue separating the muscle fibers. Bone marrow is replaced by connective tissue, which in part is finely fibrillar. The transplant is intermediate between a syngenesio- and homoiotransplant.

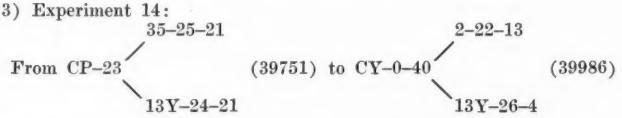
(2) Experiment 16:



Examination after 21 days. Here thyroid, cartilage, bone, bone marrow, muscle and fat tissue are relatively

well preserved; but there is a distinct increase in lymphocytes in various places and at certain points there is also an increase in connective tissue. The result corresponds to syngenesiotransplantation.

(3) Experiment 14:



Examination after 19 days. Thyroid has been destroyed and replaced by fibrous tissue and some collections of lymphocytes. Cartilage is surrounded by dense fibrous tissue and a dense mantle of lymphocytes. Bone marrow is largely replaced by connective tissue. Fat tissue is to a great extent infiltrated with epithelioid and giant cells. Homoioreaction.

In the last experiment in which a hybrid 2/13 is the host, the reaction is more pronounced than in the others; this result corresponds to analogous observations of Loeb and Wright, who likewise noticed that hybrids between families 13 and 2 react very actively against strange tissues. The number of these experiments is as yet too small for definite conclusions; but, as far as the results obtained so far go, they are of a similar kind to those found in transplantations from hybrid between two inbred families to one of the component parent families. Such a similarity in the reactions in these two series of experiments might be expected, because in both cases the host reacts against a strange set of genes present in the transplant, while another set of genes is similar or identical in both host and transplant. However, while in the transplantation from hybrid to one of the parent families, the host does not contain a set of genes which is not represented in the transplant, in the kind of transplantations which we are considering here, there is found in the host a set of genes which is strange to the donor. It is an interesting problem whether this admixture in the host alters the reaction to the transplant. In order to

decide this question it would be necessary to enlarge this series of transplantations considerably and to compare the results obtained with those in transplantation from hybrid to one of the component parent families.

Discussion

The principal conclusions which may be drawn from our experiments have been discussed at the end of the different chapters dealing with the various kinds of experiments. We shall here emphasize some of the more general conclusions. In the first place it follows from our experiments definitely that in correspondence with the chance of greater genetic dissimilarities in case we have to deal with hybrids between different families as compared with animals belonging to the same closely inbred family, the results of transplantation are less favorable in the first than in the second case. It follows, furthermore, that the results are better if the families represented in the genetic constitution of the donor are also represented in the host than when the donor contains a genetic constituent which is not present in the host. Thus in transplantation of tissues in hybrids whose parents belonged to different families, but in which the families were the same in host and donor, in a considerable number of cases results approaching autotransplantation were obtained; this was not so, when one family present in the donor was not represented in the host.

Also the result of our brother to brother transplantations confirm these conclusions. In case the brothers are hybrids between the same two inbred families the results of transplantation are slightly less favorable than in case we have to deal with brothers within the same closely inbred family, but they are always better than those of ordinary brother to brother transplantation within non-inbred families. Homoiotransplantations used as controls behaved in the usual manner and no difference could be found between homoiotransplantation

between guinea pigs derived from entirely unrelated stock and between guinea pigs which many generations back had descended from the same stock.

Thus, on the whole, our results are confirmatory of the conclusion that reactions of a host against a transplant represent a finely graded indicator of the genetic relationship between individuals and can therefore be used in the analysis of individuality differentials.

SUMMARY AND CONCLUSIONS

(1) If instead of transplanting tissues from one member of an inbred family of guinea pigs to another member of the same family, we transplant from a hybrid between two inbred families to another hybrid of the same kind the results become more unfavorable in accordance with the greater difference in average genetic constitution between host and donor to be expected under these conditions. In our experiments the most favorable results were obtained in those cases in which in host and donor the families of father as well as of mother belonged to the same generation in both hybrids. In accordance with expectation the average of the results obtained in this series of transplantations, as a whole, is much superior to ordinary homoiotransplantations and corresponds approximately to the results of ordinary (brother to brother) syngenesiotransplantation. There is also an indication that exchange of tissues between hybrids within the same family will yield somewhat more unfavorable results than exchange of tissues between strictly inbred individuals within the same family.

(2) The result of transplantations between hybrids of the same inbred families, when donor and host are brothers or sisters, is much superior to ordinary syngenesiotransplantations in non-inbred families, but is somewhat less favorable than transplantations between non-hybrid brothers belonging to the same inbred family; these results are also in accordance with differences in the genetic constitution in such animals.

(3) In accordance with expectation, transplantation from hybrids between inbred families to non-related guinea pigs either non-inbred or representing hybrids between two inbred families other than those used for the first hybridization, give ordinary homoioreactions.

(4) In addition we carried out a small number of transplantations from one of the parent strains to a hybrid between two inbred families and vice versa from a hybrid to one of the component parent strains. The former type of transplantations yielded favorable results in a former large series of experiments of Loeb and Wright. One new experiment of this kind agreed with the former ones, while in a second case an abnormal result was obtained, presumably due to an accidental error. Transplantations from a hybrid to one of the parent strains yielded results varying between syngenesio and homoio reactions in accordance with our previous results. Similar to the latter also were the results of transplantation between two hybrids in which one of the component parent families was the same, while the second parent family was different.

(5) In accordance with previous conclusions of Loeb and Wright, we may assume that the severity of the reaction against the transplant is determined by the number of strange gene-derivatives which are introduced with the transplant into the host and by the degree of their strangeness. A comparison between the results obtained in transplantations from hybrid to component parent strain on the one hand, and in transplantations between hybrids in which one family entering into the hybrid constitution is the same while the other family differs, on the other hand, if based on a large series of experiments, should answer the question whether in addition to the strangeness of the gene derivatives, introduced with the transplant into the host, the presence of strange gene derivatives in the host plays a rôle in determining the intensity of the reactions of the host against the transplant.

ON THE NATURE OF SIZE FACTORS IN MICE

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INTRODUCTION

THE nature of the factors controlling size in plants and animals has long been of interest to geneticists. In a few cases the difference between large and small size is apparently due chiefly to a single segregating gene. Such were Mendel's tall and short peas. Other examples are the "giant" mutation in *Drosophila melanogaster,* described by Bridges and Gabritschevsky (1928), and "dwarf" in mice reported by Snell (1929). More frequent are the examples in which the size of a particular part of the organism is determined by a single gene. Lindstrom (1926) found small fruit size in *Lycopersicum* partially dominant in some cases. Short-ear in mice behaves as a simple recessive to normal ear (Lynch, 1921). Another example is the "tailless" condition in mice (Lang, 1912). But still more numerous are the many instances in which size inheritance is "blending." This is the usual mode in both plants and animals.

It is now generally agreed that the genetic basis of size is prevailingly dependent on several or many genes. Davenport (1917) concluded that in humans the factors for tallness were mostly recessive. Usually, however, the multiple factors involved are without dominance and the inheritance is blending. The multiple factor hypothesis was employed by Phillips (1912, 1914) to explain the results of size inheritance experiments with ducks and by MacDowell (1914) for rabbits, Punnett and Bailey (1914) for fowls and Castle (1922) for rabbits.

Recently, however, Castle (1929) after further work on the inheritance of size (weight) in rabbits has stated that it is an open question whether general body size is determined by chromosomal genes. He was unable to detect any evidence for linkage between size and any of several

Mendelian qualitative characters affecting coat color. On the other hand, the author (1930, 1931) found indications of genetic linkage between qualitative and quantitative characters in a mouse species cross. This finding would tend to show that at least some of the size factors in mice are chromosomal.

A mooted question concerning the nature of size factors in mammals is whether they are general in their action, influencing all parts of the body as Castle (1924, 1930) believes them prevailingly to be, or whether they are specific, affecting primarily certain definite parts. Sumner (1924) believes that there are such local factors in Peromyscus, and Davenport (1930) holds the same for man. In the papers previously cited, the author stated his belief that in mice the differences in the behavior in inheritance of various quantitative characters suggested the existence of local as well as general factors for size. In the present paper it is proposed to examine statistically some of these differences in an endeavor to throw some light on the nature of size factors in mice.

Material

The material investigated in this study consists of several quantitative characters of a race of small Chinese mice, *Mus bactrianus* (the descendants of wild individuals brought from China in 1926), an inbred strain of dilute brown non-agouti *Mus musculus,* the F_1 hybrids, and a back-cross generation produced by mating *musculus* females to F_1 males. The quantitative characters considered are the following: body length, tail length, adult weight, skull length, skull width, humerus length, length of femur, transverse diameter of femur, sagittal diameter of femur, diametric ratio of femur, tibia length and cranial capacity. No mouse of a lesser age than 181 days was included in the computations, so it is probable that the size characters had attained their maximum development.

Body length and tail length were measured by a method similar to that described by Sumner (1927).

The length of the body by this method is the total length less tail length.

In a study of growth, the mice were weighed every tenth day from the thirty-first to the one hundred eighty-first day. The maximum value recorded for a mouse was considered its adult weight.

Skull length is the distance from the highest point of the foramen magnum to the most anterior surface of the incisor teeth.

Skull width is the inter-orbital width, the width of the frontal bones between the points of their strongest lateral constrictions. Because of the curvature of the frontal bones in the region adjacent to these points this dimension in the small skulled *bactrianus* could not be measured, by means of the Starrett bench micrometer employed for skeletal measurements, with as great accuracy as might be desired. Hence this measurement is considered only in the case of the larger skulled *musculus*.

Humerus length is the distance from the highest point of the head to the most distal point of the external side wall of the trochlea.

Femur length is taken as the distance from the highest point of the first trochanter to the deepest point of the internal condyle.

Transverse diameter is the least diameter, taken transversely, of the shaft of the femur between the third trochanter and the distal extremity.

Sagittal diameter is the diameter, taken antero-posteriorly, of the shaft of the femur at the level of the transverse diameter described above.

Diametric ratio of the femur equals the sagittal diameter divided by the transverse.

Tibia length is measured from the most proximal point of the articulation surface to the most distal point of the medial malleolus.

The humerus, femur and tibia measured were always the right.

The cranial capacity was determined by filling the skull with mercury, which was then weighed. Repeated measurements rarely showed deviations exceeding one half of one per cent. The approximate capacity in cubic centimeter may be obtained by dividing the values given by 13.5438 (the specific gravity of mercury at 21° C.).

Consideration of Data

Table I presents the mean values of eleven quantitative indices for *bactrianus, musculus,* and the first generation hybrids. It will be seen that the F_1 means fall into two general classes: one in which the value is intermediate between those of the parental species, and the other in which the F_1 mean equals or exceeds the mean of the larger parent race. In every instance the F_1 mean is significantly greater than that of the smaller parent. With the exception of the diametric ratio of the femur, the "larger parent" race is *musculus.*

The first class (those characters for which the deviation of the F_1 from the larger parent is greater than three times its probable error with the sign negative) includes the following: body length, tail length, adult weight, humerus length, transverse diameter of femur, sagittal diameter of femur (females) and diametric ratio of femur. The second class (those characters for which the deviation of the F_1 from the larger parent is positive in sign or less than three times its probable error with the sign negative) comprises the following: skull length, femur length, sagittal diameter (males), tibia length and cranial capacity. The F_1 males significantly exceed *musculus* males in skull length, femur length and sagittal diameter. This may be explained by the circumstances that the sex differences for these characters as well as for the diametric ratio are more marked in *musculus* than in *bactrianus* or in F_1 animals.

These two classes of F_1 means seem to show that not all factors influencing size which go into the hybrid animals from their diverse parents are general in their

TABLE I

MEAN VALUES OF QUANTITATIVE INDICES

		bactrianus		*musculus*		F_1 *musculus* ♀ × *bactrianus* ♂		Deviation of F_1 from larger parent	Deviation / P.E.
		No.	Mean	No.	Mean	No.	Mean		
Body length (mm)	♂	17	80.2 ± .51	72	95.8 ± .23	39	90.8 ± .40	− 5.0 ± .46	−10.9
	♀	26	79.7 ± .49	56	98.3 ± .50	36	91.3 ± .45	− 7.0 ± .67	−10.4
Tail length (mm)	♂	12	68.8 ± 1.22	63	93.7 ± .24	32	85.7 ± .37	− 8.0 ± .44	−18.2
	♀	21	67.7 ± .53	56	92.0 ± .38	32	85.0 ± .44	− 7.0 ± .58	−12.1
Adult weight (gms)	♂	19	15.5 ± .17	35	31.3 ± .47	37	22.0 ± .32	− 9.3 ± .57	−16.3
	♀	14	12.8 ± .23	32	30.9 ± .76	16	18.3 ± .36	−12.6 ± .84	−15.0
Skull length (mm)	♂	35	20.40 ± .050	79	21.67 ± .032	39	21.98 ± .040	+ .31 ± .051	+ 6.1
	♀	33	20.52 ± .058	93	21.89 ± .043	37	22.08 ± .059	+ .19 ± .073	+ 2.6
Humerus length (mm)	♂	19	10.44 ± .054	67	11.37 ± .020	39	11.25 ± .029	− .12 ± .035	− 3.4
	♀	29	10.23 ± .044	90	11.30 ± .026	40	11.00 ± .034	− .30 ± 0.43	− 7.0
Femur:									
Length (mm)	♂	37	13.76 ± .058	70	14.26 ± .033	39	14.67 ± .044	+ .41 ± .055	+ 7.5
	♀	37	13.74 ± .080	92	14.77 ± .046	34	14.79 ± .063	+ .02 ± .078	+ .3
Transverse	♂	42	1.24 ± .007	73	1.77 ± .009	41	1.56 ± .013	− .21 ± .016	−13.1
Diameter (mm)	♀	43	1.25 ± .010	98	1.83 ± .012	40	1.53 ± .013	− .30 ± .018	−16.7
Sagittal	♂	42	1.00 ± .007	73	1.07 ± .006	41	1.13 ± .011	+ .06 ± .013	+ 4.6
Diameter (mm)	♀	43	1.02 ± .008	98	1.25 ± .012	40	1.15 ± .013	− .10 ± .018	− 5.6
Diametric ratio	♂	42	.799 ± .005	73	.606 ± .004	41	.725 ± .006	− .074 ± .008	− 9.3
(Sagittal) / (Transverse)	♀	43	.817 ± .006	98	.681 ± .003	40	.751 ± .005	− .066 ± .008	− 8.3
Tibia length (mm)	♂	37	15.31 ± .063	67	16.53 ± .039	38	16.53 ± .050	.0 ± .063	0
	♀	37	15.17 ± .068	94	16.62 ± .048	39	16.50 ± .050	− .12 ± .069	− 1.7
Cranial capacity	♂	34	4.64 ± .034	59	5.30 ± .025	38	5.41 ± .035	+ .11 ± .043	+ 2.6
(gms of Hg)	♀	33	4.70 ± .037	70	5.33 ± .026	32	5.46 ± .037	+ .13 ± .045	+ 2.9

effects. It might be objected that the influence of heterosis is more marked in skull length and tibia length than in body length and adult weight, or in femur length than in humerus length. Just why, it would be difficult to say. But when heterosis is reduced to a purely genetic basis, it simply means an increase in the number of heterozygous factor pairs in the offspring through different dominant genes brought in by the diverse parents. Wright (1922) and Livesay (1930) have fully discussed the evidence for this view-point. Were the size factors nearly or quite general in their action there seems to be no good reason why an F_1 animal should possess a femur as long as (if a female)—or longer than (if a male)—that of the larger parent while its humerus is significantly shorter. Perhaps a result of disharmonic species crossing!

The nature and relationship of size factors can also be shown to a certain extent by means of correlations between various pairs of quantitative characters. A high coefficient of correlation for a given pair indicates that both are largely subject to common influences or factors. A low coefficient, on the contrary, tends to show that they are largely determined by different factors. Castle (1914, 1922, 1930) has made a number of such correlations for rabbits and found them uniformly high; thus, he believes, demonstrating that size factors in rabbits are, for the most part, general. Wright (1918) in an analysis of Castle's (1914) data by partial correlations has shown that even with the high coefficients of correlation there are factors which influence definite parts of the body independently of general size.

In Table II are presented coefficients of correlation for twelve pairs of characters for each sex of *musculus,* three pairs for *bactrianus,* and the same three pairs for an F_1 generation and for one type of back-cross mating. The types of matings and the two sexes are treated separately since, as Wright pointed out, the inclusion of heterogeneous material would tend to overemphasize the importance of general factors for size.

TABLE II

COEFFICIENTS OF CORRELATION

	Correlations	♂♂ No.	♂♂ Value of r	♀♀ No.	♀♀ Value of r
	musculus ♀ × *musculus* ♂				
1.	Skull length—body length	78	+.601 ± .049	60	+.745 ± .038
2.	" " —body weight	34	+.401 ± .097	28	+.360 ± .111
3.	" " —humerus length	76	+.365 ± .067	86	+.635 ± .043
4.	" " —femur length	68	+.323 ± .073	81	+.602 ± .048
5.	" " —tibia length	75	+.424 ± .062	90	+.538 ± .051
6.	" " —skull width	79	+.386 ± .065	93	+.397 ± .059
7.	Humerus length—body length	83	+.447 ± .059	59	+.724 ± .042
8.	" " —body weight	33	+.220 ± .112	28	+.615 ± .080
9.	" " —femur length	72	+.627 ± .049	88	+.788 ± .027
10.	" " —tibia length	81	+.656 ± .042	98	+.703 ± .034
11.	Femur length—tibia length	70	+.761 ± .034	92	+.818 ± .024
12.	Body length—body weight	35	+.395 ± .096	32	+.653 ± .068
	bactrianus ♀ × *bactrianus* ♂				
13.	Skull length—femur length	32	+.249 ± .112	31	+.637 ± .072
14.	Skull length—tibia length	35	+.265 ± .106	33	+.630 ± .071
15.	Femur length—" "	37	+.817 ± .037	37	+.855 ± .030
	musculus ♀ × *bactrianus* ♂				
16.	Skull length—femur length	39	+.291 ± .099	32	+.335 ± .106
17.	" " —tibia length	38	+.415 ± .091	36	+.407 ± .094
18.	Femur length—" "	38	+.657 ± .062	32	+.578 ± .079
	musculus ♀ × *musculus* ♂ / *bactrianus*				
19.	Skull length—femur length	63	+.546 ± .060	51	+.473 ± .073
20.	" " —tibia length	70	+.723 ± .038	65	+.284 ± .077
21.	Femur length—" "	63	+.916 ± .014	52	+.857 ± .025

Although the numbers are small in some cases—in consequence of which the probable errors are large—it is nevertheless apparent that there are significant and important differences in the values of the coefficients of correlation between various pairs of characters. Skull length and body length, for example, are more closely correlated than skull length and skull width. Femur length and tibia length are most closely correlated in all types of matings. Castle (1922) found the value of r for femur length-humerus length in rabbits to be .91; for tibia-humerus, .90; for femur-tibia, .93. In our *musculus* matings the femur-tibia correlation is consistently—although not in every case significantly—higher than the other two.

TABLE III

COEFFICIENTS OF CORRELATION IN PARENT SPECIES AND HYBRIDS

	1 Femur length—tibia length r	2 Femur length—skull length r	3 Tibia length—skull length r	Difference 1–2	Difference / P.E.	Difference 1–3	Difference / P.E.
musculus ♂	+.761 ± .034	+.323 ± .073	+.424 ± .062	.438 ± .081	5.4	.337 ± .071	4.7
♀	+.818 ± .024	+.602 ± .048	+.538 ± .051	.216 ± .054	4.0	.280 ± .056	5.0
bactrianus ♂	+.817 ± .037	+.249 ± .112	+.265 ± .106	.568 ± .118	4.8	.552 ± .112	4.9
♀	+.855 ± .030	+.637 ± .072	+.630 ± .071	.218 ± .078	2.8	.225 ± .077	2.9
F_1 ♂	+.657 ± .062	+.291 ± .099	+.415 ± .091	.366 ± .177	3.1	.242 ± .110	2.2
♀	+.578 ± .079	+.335 ± .106	+.407 ± .094	.243 ± .132	1.8	.171 ± .123	1.4
Back-cross ♂	+.916 ± .014	+.546 ± .060	+.723 ± .038	.370 ± .062	6.0	.193 ± .040	4.8
♀	+.857 ± .025	+.473 ± .073	+.284 ± .077	.384 ± .077	5.0	.573 ± .080	7.2

TABLE IV

SEX DIFFERENCES IN *musculus* ♀ × *musculus* ♂ MATINGS

Correlations	Value of r ♂♂	Value of r ♀♀	Difference	Difference / P. E.
Skull length-humerus length	+.365 ± .067	+.635 ± .043	.270 ± .080	3.4
" " -femur length	+.323 ± .073	+.602 ± .048	.279 ± .087	3.2
Humerus length-body length	+.447 ± .059	+.724 ± .042	.277 ± .072	3.8
" " -body weight	+.220 ± .112	+.615 ± .080	.395 ± .138	2.9
" " -femur length	+.627 ± .049	+.788 ± .027	.161 ± .056	2.9

When the femur-tibia correlations are compared with femur-skull and with tibia-skull it is evident that the coefficients for the first pair are much higher than the values for the latter two. This is shown in Table III. This seems to demonstrate that the factors determining skull length in mice are not entirely the same as those determining the length of femur or tibia; while the two leg bones do form a group largely subject to the same genetic influences. This is in line with Wright's analysis (cited above) of Castle's rabbit material.

In Table I it was shown that there were significant sex differences in the means of several of the size characters investigated. Female *musculus* are significantly longer bodied than males and have longer skulls and longer femurs with greater transverse and sagittal diameters. The diametric ratios of the females also average higher. There are likewise sex differences in the degree of correlation between various pairs of characters. Of the twelve pairs of correlated characters in *musculus,* eleven show higher coefficients of correlation for females than for males. In Table IV are listed five pairs for which the differences approach significance. This tendency may mean that in female mice general size factors have more influence than they do in males. Merriam (1896) in a taxonomic revision of the weasels of North America stated that among mammals the female is less specialized than the male and consequently bears more resemblance to the ancestral stock. Perhaps in mice specialization also extends to genetic size factors.

SUMMARY

Data on quantitative characters, derived from a mouse species cross, have shown that the F_1 means fall into two general classes: one in which the means are intermediate between those of the two parent species and the other in which they equal or surpass the larger parent. In general, external dimensions (body length, tail length) and weight fall into the first; while skeletal dimensions fall

into the second. Humerus length, however, is intermediate. These results make it appear probable that not all size characters are equally subject to the influence of general factors.

Coefficients of correlation were computed for various pairs of characters and disclosed significant differences. Femur and tibia are more closely correlated than is either with skull length. These two leg bones in mice, as well as in rabbits, are apparently largely influenced by common factors. Sex differences were found in the degree of correlation between various pairs. In general, females present higher coefficients than males perhaps indicating that general size factors play a more important rôle in the former.

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A MUTUAL TRANSLOCATION INVOLVING THE FOURTH AND THE X-CHROMOSOMES OF DROSOPHILA

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INTRODUCTION

NUMEROUS instances of broken chromosomes and translocated fragments have been reported. Cases described previously for Drosophila have involved breakages of the X-, Y-, II- and III-chromosomes. This paper presents evidence of the first demonstrated breakage of the IV-chromosome.[1]

ORIGIN OF STOCK

The stock, known as mottled 5, was produced by Dr. Muller in 1929 by X-raying a male carrying a normal X-chromosome. It was inferred to involve a breakage and translocation of the X to an autosome on account of flies of characteristic hyperploid appearance produced by it (Muller, 1930). Males possessing the treated X-chromosome exhibit mottled eyes. Females show the mottledness when they have, in addition to the treated chromosome, the gene for white in the other X-chromosome. The genes for vermilion and forked were subsequently placed in the treated chromosomes by crossing-over. The $w^{m5}vf$ strain has been used almost exclusively in these experiments because of the convenient markers, vermilion and forked.

ANALYSIS OF STOCK

(a) *Translocation of X-fragment to IV*

Since the mottledness of the eyes never appears in females heterozygous for the normal allelomorph of white, but does show if a light allelomorph (*e.g.*, white or eosin) is present, it is evident that the locus of white

[1] I express my thanks to Dr. H. J. Muller for suggestions and guidance in attacking this problem.

in the X-chromosome is the one primarily involved in the mottling. Tests show that mottledness is linked to the IV-chromosome. When w^{m5} females were mated to bent males and the F_1 females backcrossed to bent males, both mottled males and bent males appeared in the F_2 but there were no mottled bent males. Subsequent tests proved the loci of yellow and scute were likewise linked to IV but that facet and miniature were not.

Hyperploid males were produced by mating $w^{m5}vf$ males to yw females. From a few such crosses there were obtained a total of 237 gray mottled females, 203 yw males and 45 gray mottled males. The F_1 mottled males undoubtedly received their X-chromosome from the female parent. Had they been non-disjunctional progeny they should have shown vermilion and forked, for these characters could not have been eliminated by crossing-over in the male parent. Hence the mottled males carry an entire yw X-chromosome. But inasmuch as they are gray and mottled they also contain a portion of an X which includes the loci of yellow (gray) and white (mottled). This fragment could have come only from the male parent. Obviously these mottled males are hyperploid.

Some of the hyperploid males show abnormal venation, usually a projection on the posterior crossvein; the hind legs are sometimes deformed; often the wings are spread. The viability is about 20 to 25 per cent. of the yw males. In these respects they agree with other hyperploids carrying as excess the left end of the X. The hyperploid males breed although the fertility is less than normal.

Hyperploid females may be obtained and recognized by backcrossing the hyperploid males described above to yw females. One such cross yielded the following offspring: 97 yw females, 73 yw males, 49 gray mottled females and 20 gray mottled males. The yw flies are non-hyperploid; the gray mottled ones have received the extra fragment. Some of the hyperploid females exhibit venation disturbances.

Hyperploid males obtained by mating w^{m5}vf males to yellow white facet females are gray mottled facet; those from yellow scute white miniature females are gray non-scute mottled miniature. The conclusion is that the X-chromosome is broken between the loci of white and facet and that the left end is attached to the IV-chromosome. The translocation has been termed "X–IV2," as it is the second case of breakage of the X and its attachment to chromosome IV. For the first case see Muller and Stone (1930) and for the third case Patterson and Painter (1931).

(b) *Translocation of IV-fragment to X*

The w^{m5}vf males were mated to yellow attached-X eyeless females. The F_1 w^{m5}vf males were backcrossed to yellow attached-X eyeless females. The F_2 females were yellow eyeless, gray eyeless, yellow non-eyeless and gray non-eyeless. The F_1 males are all non-eyeless. The gray females carry the IV to which the left end of the X is attached as shown by the fact that they are non-yellow. Two alternative explanations of the presence of eyeless in part of the gray females arise. The first is that a mutation to eyeless has occurred in the IV to which the X-fragment is attached. This is obviated by the fact that the w^{m5}vf males are all non-eyeless.

The second alternative assumes that the IV-chromosome is broken and that a portion carrying the normal allelomorph of eyeless is attached to the right end piece of the broken X. This will account for the eyeless condition of some of the F_2 gray females mentioned above. They had received an eyeless IV from the mother and the IV carrying the X-fragment from the father. If the locus of eyeless is absent from this paternal IV, then the appearance of eyeless in the F_2 gray females is to be expected. The F_2 w^{m5}vf males are all non-eyeless because they possess, in addition to the part of the IV to which the X-fragment is attached, that portion of the IV containing the normal allelomorph of eyeless. This frag-

ment of the IV can not be attached to the Y-chromosome, for the Y-chromosome came from a mother of normal IV ancestry. If it were attached to either of the large autosomes only half of the F_2 $w^{m5}vf$ males would have received it and have been non-eyeless. Therefore the IV-fragment must be attached to the right portion of the X-chromosome but to the left of vermilion, for vermilion and forked got into the chromosome by crossing-over after the x-ray treatment.

(c) *Non-disjunction of IV*

In the F_1 of the cross discussed above all the flies were non-eyeless although the gray females might be expected (on the present hypothesis) to show eyeless. When these F_1 gray females were mated to y sc w ey males the progeny were as follows: yellow, yellow eyeless, gray, gray eyeless females and yellow scute white, yellow scute white eyeless, gray mottled, gray mottled eyeless males. It is evident that some of the gametes carrying the gray mottled containing fragment must also have carried the normal allelomorph of eyeless. This has occurred through non-disjunction of the IV. The fragmented and attached condition of the IV probably would hinder perfect synapsis and result in a high degree of non-disjunction. Apparently an eyeless fly which receives only one entire IV and the fragment of the IV not containing the locus of eyeless has low viability.

Various crosses were designed and carried out which should have given eyeless flies in the F_1 if IV-hypoploid eyeless flies are viable, but no eyeless offspring appeared. Special effort was made to furnish optimal cultural conditions for the test. Yellow attached-X eyeless females were mated to $w^{m5}vf$ males. From three cultures there were obtained 81 $w^{m5}vf$ males, 74 yellow females and 46 gray females. None of the gray females was eyeless although all had received an eyeless IV from the mother and from the father the portion of the IV which lacks the locus of eyeless. It seems evident that through non-dis-

junction a normal IV had accompanied the IV-fragment from the male parent. When w^{m5}vf males were crossed to y sc w ey females the following progeny were secured from ten cultures: 381 gray mottled females, 253 y sc w males and 57 gray mottled males. Again, the failure of gray mottled eyeless males to appear indicates a very low viability (perhaps inviability) of eyeless flies haploid for the portion of the IV containing the locus of eyeless.

There is cytological evidence of non-disjunction of IV in mottled 5. Dr. Bessie League (unpublished data) found some cells containing what were apparently three IV-chromosomes, and other cells with four. The translocated X-fragment has not been detected, presumably due to its small size. In this connection compare the case reported by Patterson and Painter (1931).

Discussion

Flies hypoploid with respect to the X-chromosome have not been discovered in the w^{m5} stock. When w^{m5}vf males are mated to yw females all the female offspring are gray mottled. Females lacking the left end fragment of the X do not live even though they possess one complete X-chromosome. This harmonizes with Patterson's demonstration of a viability gene in the left end of the X-chromosome (Patterson, in press).

The evidence indicates that the IV-chromosome is broken between the locus of eyeless and the point of attachment of the spindle fiber. I propose to refer to the locus of eyeless as being to the left of the spindle fiber. It seems clear that in the left end fragment there is a gene or genes, the double dose of which is important for the viability of the fly, at least when the latter has the gene for eyeless and not for its normal allelomorph.

Tests with bent similar to those performed with eyeless indicate that it is in the right end portion of the IV. Other fourth chromosome genes may be tested to determine their locations with respect to the break and thus

a partial map of the IV-chromosome may be constructed. As shown by work of Morgan, Sturtevant and Bridges (1926), and of Patterson and Muller (1930, see pp. 523–524) previously published "maps" of this chromosome, based upon supposed crossing over (Morgan, *et al.* 1925) are invalid.

SUMMARY

(1) The w^{m5}vf stock of *Drosophila melanogaster* contains a mutual translocation of X and IV, produced by x-ray.

(2) The X-chromosome is broken between white and facet; the left end is attached to the IV.

(3) The IV-chromosome is broken between eyeless and bent and the eyeless (left) part is attached to the right portion of the broken X. Bent is located on the spindle fiber (right end) portion of the IV.

(4) Non-disjunction of IV occurs very frequently in this stock.

(5) A gene, the double dose of which is important for viability, is located in the left (eyeless) end of the IV.

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THE WEIGHT–LENGTH RELATIONSHIP OF THE SHELLS OF THE HAWAIIAN PEARL OYSTER, *PINCTADA SP.*[1]

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SINCE the formulation of Spencer's "cube law" (1872) stating that the weights of the organisms increase as the cubes of their dimensions, numerous attempts were made to apply this relation to various animals and to fit the observed and calculated data to a simple formula, $W = aL^3$, where W is weight, L is length and a is a constant dependent on the unit of measure and the organism studied. This simple formula was often used in a study of growth and in comparing the weight-length factors, $a = \frac{W}{L}$, of various food fishes. An extensive and widely scattered bibliography on the subject compiled by Clark (1928) can be found in her paper on the weight-length relationship of the California sardine. Although the application of a "cube law" gave satisfactory results for fishery studies (Hensen, 1899; Heincke, 1908; Johnstone, 1914), its validity and theoretical significance for the interpretation of the relation between growth and form has been but little studied. In 1916 Hecht, accepting the cube law as representing the true relation between the weight and the length, came to the conclusion that in the organisms in which growth is uniform but unlimited (indeterminate) the form of the individual is laid down very early in life and is adhered to throughout its period of growth which lasts for the rest of its life (fishes, bivalve mollusc Dosinia), whereas in the organisms with determinate growth (man) the form is continually changing during the period of growth. In the latter case when the form becomes constant the growth ceases. In recent years Huxley (1924, 1927) has developed this idea more

[1] Published by permission of the United States Commissioner of Fisheries.

elaborately and arrived at the general formula which describes both the "isogonic" growth, when the part bears a constant relation to the whole, and the "heterogonic" growth when a part of the body is increasing or decreasing in relation to the whole.

The works of Clark (1928), Duncker (1923), Keys (1928) have shown that the cube law fails to express the exact weight-length relationship and that the latter could be described by a more general equation $W = aL^n$ where the value of n, computed from the measurements, is ordinarily greater than 3 and less than 4. The applicability of this formula to the weight-length relationship in molluscs Sphaerium, Littorina, Limnaea and Viviparus has been demonstrated by Nomura (1926).

In July–August, 1930, the author collected 164 live specimens of adult pearl oyster, *Pinctada sp,*[2] at Pearl and Hermes reef, a small atoll of the Hawaiian archipelago located between the latitudes 27°45′ and 27°58′N and longitudes 175°43′ and 176°00′W. Besides adult oysters, varying in size from 10 to 29 centimeters, the collection comprised 33 small oysters from 0.3 to 3.5 centimeters long which were found attached to the shells of the adult ones. They undoubtedly represented the newly hatched oysters that have just attached themselves. Oysters of intermediate size, from 6 to 10 centimeters long, were absent. This suggests that the spawning of the pearl oyster in Pearl and Hermes reef occurs only during the summer. Physiological and ecological observations which will be published elsewhere corroborate this conclusion.

Inasmuch as the rate of growth of the oyster is not known, there is no means of determining the age of the

[2] In the National Museum in Washington and in the Bishop Museum at Honolulu the Hawaiian pearl oyster is listed under the name *Pinctada cumingi* Reeve. A careful examination of the material collected in the Hawaiian Islands has convinced me that the Hawaiian species differs from the characteristics given by Reeve and referring to the oyster from the Paumotu Islands. A detailed description of the Hawaiian species will be given elsewhere.

individuals of various sizes. It is very likely, however, that the one year old oyster is about 13 centimeters long. This conclusion is based on the measurement made on one specimen which was planted in the summer of 1929 by one of the divers employed by the author, and was found again in August, 1930. According to the testimony of the diver the oyster, when planted, was about one inch long. During July and August, 1930, all the oysters of 10 centimeter size or larger contained fully developed gonads and were ready to spawn.

In the following discussion the length, L, is regarded as the greatest dimension of the shell measured at a right angle to the hinge side. The width, W, is the greatest distance across the shell at a right angle to the length. Both length and width were measured to the nearest half centimeter. The weight of the shells, dried at room temperature, was determined to the nearest gram. Figures of weight, given in the text, indicate the weight of both valves of the oyster. In measuring and weighing

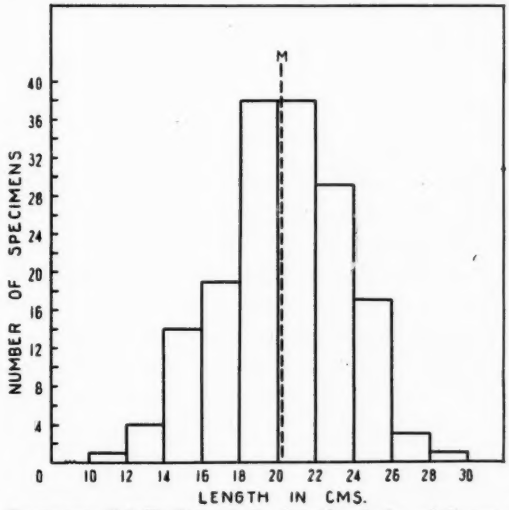

FIG. 1. Frequency distribution of the length of the adult pearl oysters. (Small oysters less than 6 cms long are not included.)

small oysters the second decimals of a centimeter or of a gram were read.

The frequency distributions of the length and weight of the adult oysters (Figures 1 and 2) indicate that specimens between 18 and 22 cms long and weighing from 400 to 600 grams were predominant. It is interesting to note that the frequency distribution of the length (Fig. 1) gives a more symmetrical curve than that of the

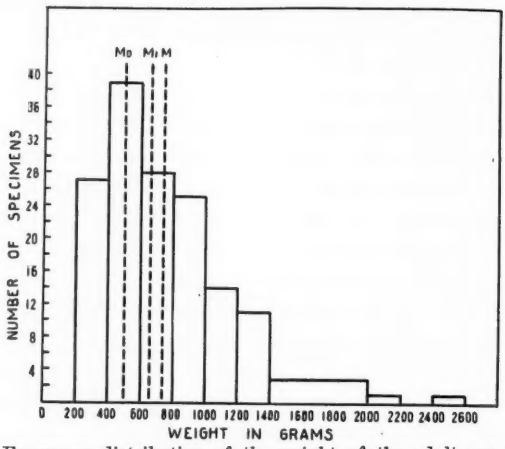

FIG. 2. Frequency distribution of the weight of the adult pearl oysters. (Small oysters less than 6 cms long are not included.)

weight (Fig. 2). The probable reason of such a difference will be discussed later.

The biometrical constants for the length and weight of the shells of the adult oysters are given in Table I.

TABLE I

BIOMETRICAL CONSTANTS FOR THE LENGTH AND WEIGHT OF THE SHELLS OF THE ADULT *Pinctada sp.*

	Mean	Pr. err.	St. deviation	Median	Mode
Length cms	20.2	± 0.17	3.24 ± 0.12	20.3 ± 0.21	20.5
Weight gms	734	± 22.6	418 ± 15.6	650 ± 28.2	492.6

A frequency distribution of the weight of the adult oysters grouped in six classes according to their length

(Fig. 3) shows that the skewness of the variation curves increases with the increase of length (compare the classes marked 21, 23 and 25 with those of 15, 17 and

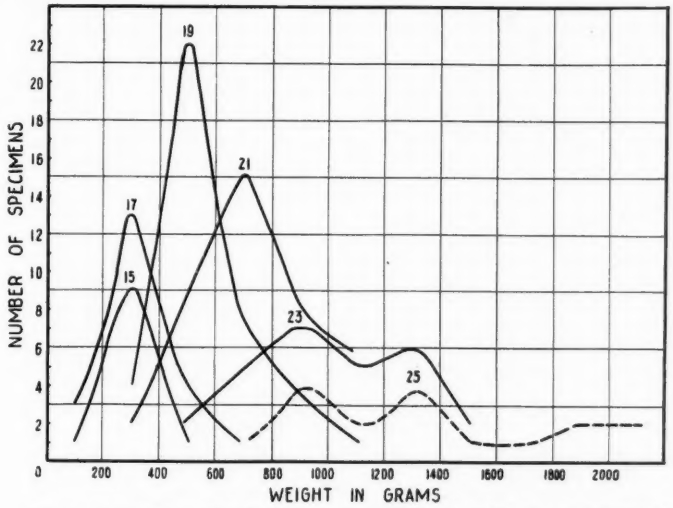

FIG. 3. Frequency distribution of the weight of shells in six classes of pearl oysters of various lengths. The figures in the body of the graph indicate the length of shells in cms. Because of a small number of observations the shape of the curve of class 25 (dotted line) can not be regarded as significant.

19). This fact, coupled with the observation that the frequency curve of the weight of the oysters (Fig. 2) is less symmetrical than the frequency curve of their length (Fig. 1), can be interpreted as indicating that the deposition of shell substance, or in other words, the increase in the thickness of the shell, continues in spite of the retardation or cessation of the growth of shell in length.

The biometrical constants for the weight of the oysters of various classes are given in Table II.

In computing the mean for the class of oysters 14–15.9 cms long one measure of 697 grams was rejected because its deviation from the mean was 10.3 times the standard error. The probable error of the mean of the class of

TABLE II

BIOMETRICAL CONSTANTS FOR THE WEIGHT OF VARIOUS CLASSES OF *Pinctada sp.*

Length cms.	Number of specimens	Mean grams	Probable error	St. deviation	Median	Mode	Coef. of variation per cent.
0– 1.9	22	0.21	± 0.02	0.15 ± 0.02	0.20 ± 0.03	0.20	66.1
2.0– 3.9	10	1.33	± 0.14	0.61 ± 0.09	1.17 ± 0.17	0.85	45.9
4.0– 5.9	1	(3.5)					
10.0–11.9	1	(68)					
12.0–13.9	4	163	± 17.5	37.5 ± 8.9			23.0
14.0–15.9	14	262	± 15.9	81.4 ± 10.8	258 ± 19.9	250	31.1
16.0–17.9	19	375	± 17.3	109 ± 11.9	356 ± 21.7	318	29.1
18.0–19.9	38	570	± 22.3	201.5 ± 15.6	522 ± 27.6	426	35.4
20.0–21.9	38	738	± 23.1	208 ± 16.1	729 ± 28.5	711	28.9
22.0–23.9	29	998	± 36.8	289 ± 15.6	925 ± 46.2	779	29.0
24.0–25.9	17	1265	± 60.8	361 ± 41.8	1275 ± 76.1	1295	28.8
26.0–27.9	3	(1849)					
28.0–29.9	1	(2509)					

Mean computed from a small number of observations is shown in parentheses.

12–13.9 cms was corrected for the small number of observations ("Student," 1925). An examination of the columns of standard deviations and of the Pearsonian coefficient of variation reveals the extent of variation in weight within each class. The coefficient of variation of small oysters (first two classes) is much higher than in the adult ones, but the differences in the coefficients of variation of larger oysters can not be regarded as significant. The highest variability probably corresponds to the period of most rapid growth (Weymouth, 1931) which, judging from our knowledge of the growth of the edible oyster (*Ostrea*), occurs immediately after the attachment of the larvae.

In spite of a great variability of weight within each class the mean weight of the oysters increases at a definite rate with the increase in length. Excepting the first class (oysters less than 2 cms long) and the two largest

classes (from 26 to 29.9 cms long), which are represented by a very small number of observations, the relation between the logarithm of weight and the logarithm of length can be represented by a straight line (Fig. 4).

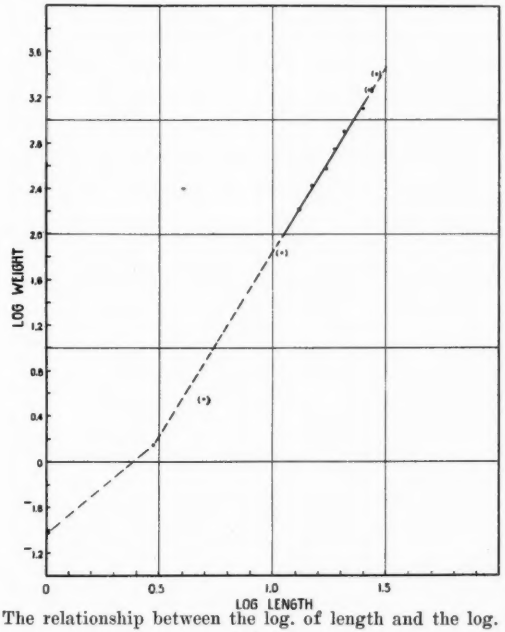

FIG. 4. The relationship between the log. of length and the log. of weight of shells.

Mathematical analysis of the curve reveals that within the limits just mentioned the weight-length relationship can be described by the formula $W = aL^n$. Determining the values "a" and "n" by the method of least squares we arrive at the following equation:

$$W = 0.04209\, L^{3.21529} \qquad (1)$$

The curve representing this equation is shown in Fig. 5. As one can notice from an examination of Fig. 5 and Table 3, whenever there is a sufficient number of observations, the observed mean values (triangles) fit the computed figures (circles) very well.

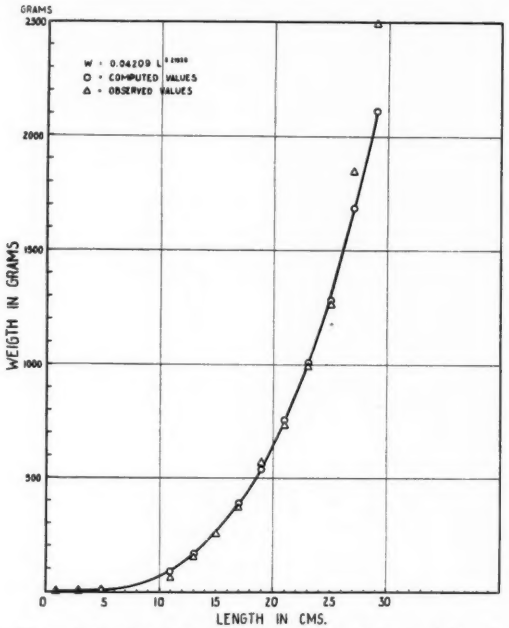

FIG. 5. The relationship between the length and the weight of shells.

TABLE III

OBSERVED AND CALCULATED VALUES OF THE WEIGHTS OF THE SHELLS OF *Pinctada sp.*

Length in cms.	Number of specimens	Observed mean weight grams	Calculated weight grams	Difference between calculated and observed weight
0.0– 1.9	22	0.21	0.04	– 0.17
2.0– 3.9	10	1.33	1.44	+ 0.11
4.0– 5.9	1	3.5	7.4	+ 3.9
10.0–11.9	1	68.0	93.9	+ 25.9
12.0–13.9	4	163.0	165.6	+ 2.6
14.0–15.9	14	262.0	254.5	+ 2.5
16.0–17.9	19	375.0	380.5	+ 5.5
18.0–19.9	38	570.0	544.1	– 25.9
20.0–21.9	38	738.0	750.7	+ 12.7
22.0–23.9	29	998.0	1,005.7	+ 7.7
24.0–25.9	17	1,265.0	1,331.7	+ 66.7
26.0–27.9	3	1,849.0	1,684.5	– 165.5
28.0–29.9	1	2,509.0	2,110.9	– 388.1

From the fact that during the period of growth from 2 to 25.9 cms the mean weight of the shell bears a constant relation to its length the following inferences can be made: that within this range the growth is not accompanied by a change in form, or if there is any change in form it is counterbalanced by the change in the specific gravity of the shell material. The second assumption is very improbable and is not confirmed by the examination of the shells. Inasmuch as the shell of the pearl oyster is flat and round the changes in its form could be easily expressed by the length-width ratios or by the percental width ($100\frac{W}{L}$). Table 4 comprising these data shows that no significant changes occur in the shape of the

TABLE IV

MEAN VALUES OF THE LENGTH-WIDTH RATIOS AND PERCENTAL WIDTH OF THE SHELLS OF *Pinctada sp.*

Length cms	Number of specimens	Mean ratio $\frac{L}{W}$	$100\frac{W}{L}$
0.0– 1.9	22	1.015±0.02	98.5
2.0– 3.9	10	1.02 ±0.03	98.0
4.0– 5.9	1	(1.05)	(95.2)
10.0–11.9	1	(1.05)	(95.2)
12.0–13.9	4	1.16 ± 0.02	86.2
14.0–15.9	14	1.17 ± 0.02	85.5
16.0–17.9	19	1.10 ± 0.01	91.2
18.0–19.9	38	1.17 ± 0.02	85.5
20.0–21.9	38	1.13 ± 0.02	88.5
22.0–23.9	29	1.19 ± 0.02	84.0
24.0–25.9	17	1.22 ± 0.03	82.0
26.0–27.9	3	(1.15)	(87.0)
28.0–29.9	1	(1.28)	(78.1)

adult oysters (from 10 to 23.9 cms long). It has been found that oysters of the class 24–25.9 cms were distinctly longer (ratio 1.22), but unfortunately an insufficient number of measures in the next larger classes makes it impossible to decide whether the difference is

significant. Small immature oysters are decidedly shorter than the adults (ratios 1.015–1.02). This confirms the conclusion reached from the examination of the weight-length curve that during the period of growth the young oysters change in shape. After they have reached 10 cms in length their form remains nearly constant.

From the analysis of the weight-length relation of shells of the Hawaiian pearl oyster a conclusion can be reached that there is no sharply defined difference between the determinate and undeterminate growth and that the growth of immature oysters is accompanied by a gradual change in their form.

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THE YAQUINA OYSTER BEDS OF OREGON

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IN connection with an investigation of the oyster beds of the Yaquina Bay and River regions of Oregon certain general biological features were revealed which are of interest to students of oyster culture, particularly those who have considered the oyster producing areas of the Pacific Coast. The investigations referred to were conducted chiefly during the fall and spring months of 1927–1928, and embraced that portion of the Yaquina River between the towns of Newport and Elk City, a stretch of over twenty miles. Since then, visits have been made at irregular intervals to this vicinity, and they have revealed that with minor differences conditions at the present time are much the same as when the intensive investigations were conducted some three years ago.

THE YAQUINA RIVER

The Yaquina River is a small, narrow stream lying about 100 miles south of the Oregon-Washington boundary, running for a distance of about thirty or more miles inland, beginning at its mouth on the Pacific Ocean and passing along its course the settlements of Newport, Yaquina, Oysterville, Toledo and Elk City. Newport is situated at the mouth of the river and Toledo is approximately eleven miles distant from it. Between these two places and for another mile above the city of Toledo, the stream is really an estuary (Fig. 1).

As mentioned, the river is narrow, and it varies in width from about 250 yards at Toledo to nearly 700 yards below Yaquina, where it enters Yaquina Bay, upon which the main part of the city of Newport is located. Above Toledo, the stream becomes much narrower, so much so that around Elk City it is no more than 100 feet across. Moreover, its main channel is quite shallow, varying in

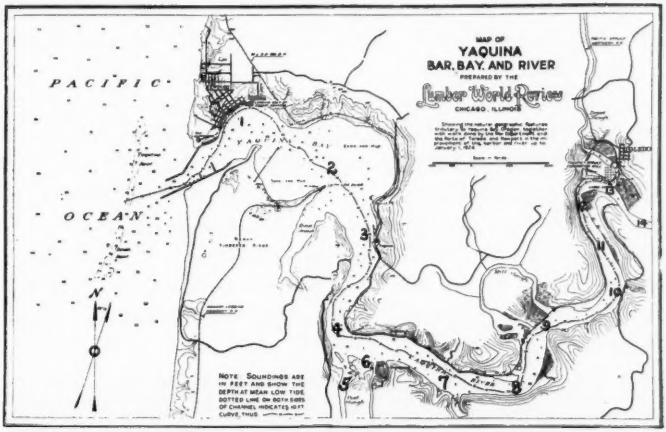
PACIFIC
OCEAN
MAP OF
YAQUINA
BAR, BAY, AND RIVER
PREPARED BY THE
Lumber World Review
CHICAGO, ILLINOIS
NOTE. SOUNDINGS ARE IN FEET AND SHOW THE DEPTH AT MEAN LOW TIDE. DOTTED LINE ON BOTH SIDES OF CHANNEL INDICATES 40 FT. CURVE, THUS

FIG. 1. Map of Yaquina River region.

depth from approximately ten to thirty feet. The river flow is controlled by the ocean tides, the average difference between the rise and fall of the waters being around eight feet. This tends to scour and cleanse the stream daily. The banks contain large quantities of sand and mud, and owing to the tides these materials are being shifted around constantly. The mouth entrance to the Yaquina River is forever changing, so much so that on all maps issued by the United States Coast and Geodetic Survey the following caution is invariably given: "The entrance to Yaquina Bay is changing. Strangers should not attempt to enter without a pilot."

The shores along the river bank are quite steep, in many places rising to a height of over 500 feet. They are covered with vegetation, sand, gravel and stone. Rains and storms, which are especially prevalent here during the fall, winter and early spring months, wash large quantities of leaves, twigs, trees, shrubs and land deposits into the waters, thereby adding to the quantities of solid particles brought down from the upper parts of the stream with the ebb tide and washed up from the ocean below with the flood tide. The stream is thereby filled with tremendous quantities of floating silt, mud and

débris, so that the ebb and flow of the tides create currents which carry these suspended particles up and down the river bed, changing and shifting it accordingly. This has been going on for years, and it has made it difficult to maintain channels for navigation. Furthermore, it has interfered with the natural oyster beds located at the small settlement of Oysterville, approximately six miles below Toledo, mainly on the south shores of the river, and extending for a distance of about three miles in a direction towards the mouth of the stream. This is shown on map, Fig. 1, between stations 3 and 8. Fig. 2 shows their exact location.

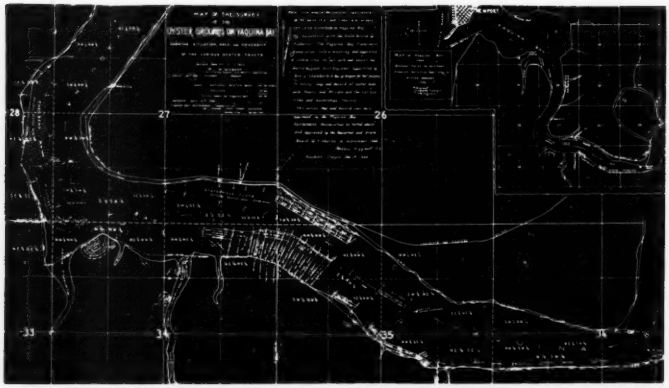

FIG. 2. Map of Yaquina oyster beds as surveyed December 1, 1908.

That this has been the case is a matter of official record, for in the year 1917, Charles H. Edmondson, special investigator for the U. S. Bureau of Fisheries, visited the region and has written a bulletin entitled "Shellfish Resources of the Northwest Coast of the United States," Appendix III to the Report of the U. S. Commissioner of Fisheries for 1922, in which he tells us the following regarding the oyster beds of the Yaquina: "Since the beds are, for the most part, located in the channel of the river, in 1917 the fishermen on the artificial plantations were having considerable difficulty, as

had been the case in previous years, in preventing the oysters from being smothered by the sediment carried down the river. Frequent tonging was resorted to to keep the silt moving. Little or no artificial methods were resorted to in order to catch the spat. The old shells were considered, by those engaged in the industry, as sufficient cultch with the occasional help of the bark of trees."

During one of my visits to this region, on November 16 and 17, 1927, the Yaquina River between Newport and Toledo was so muddy that it was completely turbid, brownish and densely opaque in appearance. The reason for this state of affairs was the terrific storm which visited the vicinity the previous day, Tuesday, November 15, when a fierce gale, blowing about seventy miles an hour, coupled with torrential rains, washed tremendous quantities of material from the shores into the stream. As already mentioned, during the late fall, winter and spring months quite a number of similar storms occur in this region so that the Yaquina River often takes on such an appearance. Large quantities of mud, silt, ooze, leaves, branches, bark, pebbles and other débris are thereby scattered far and wide, back and forth over the stream.

Into this stream, also, human sewage enters at various points. The city of Toledo empties practically all of its sewage, human and otherwise, directly into Depot Slough and from here it ultimately finds its way into the Yaquina River. The main business section of Newport dumps all of its sewage and other waste into Yaquina Bay, from where it is swept back and forth over the course of the river. Between Newport and Toledo there are smaller settlements such as Yaquina and Oysterville, and isolated boat houses as well as shore homes, which also pollute the stream with human sewage. Above Toledo a similar condition prevails, with the result that large quantities of untreated human sewage and other wastes of one kind and another, particularly from dairies, garages and boat-oiling stations, find their way di-

rectly into the stream. The tides then carry such material back and forth over the entire river, making it possible for dangerous disease-producing organisms to be disseminated to those forms which dwell in the stream and are utilized by man for food purposes. Inasmuch as the oysters from the Yaquina region are sent to various markets, mainly those of the Portland district, for human consumption, they may be regarded as a source of grave danger to human individuals.

GENERAL EXTENT OF OYSTER BEDS

The oyster beds on the Yaquina River are located around the settlement of Oysterville, situated mainly on the south banks of the stream, beginning a little above the town of Yaquina and extending for a distance of about three miles towards Toledo. Fig. 2 is a photograph of an official map of the oyster beds as they existed in 1908, the last time that they were surveyed and recorded. Fig. 3 shows the shore of Oysterville as it ap-

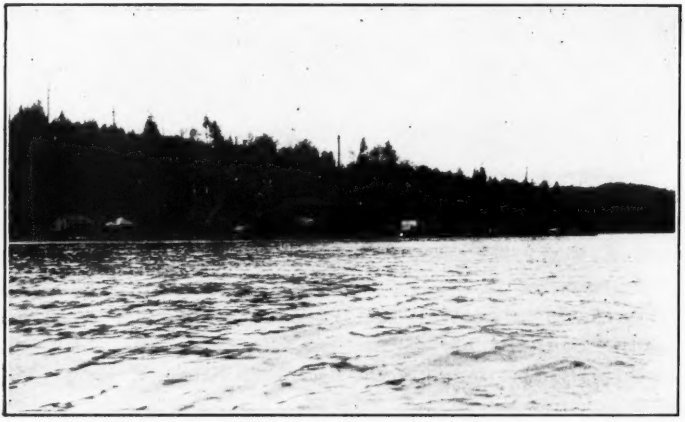

FIG. 3. Shore at Oysterville.

pears at the present time. Eastern oysters have been planted here during the last few years.

Harlan B. Holmes, of the U. S. Bureau of Fisheries, who investigated these oyster beds in January, 1927, and

has written an unpublished report on them, describes their location as follows: "Along the lower four or five miles of the river, from near the town of Yaquina to Boon's Point, there are approximately 135 acres of oyster beds. The oysters are of a native variety which is somewhat larger than the well known Olympia oyster of Puget Sound. A part of the beds are the property of the State of Oregon. The remainder formerly were owned by a number of individuals, but several years ago a company known as the Oregon Oyster Company acquired title to all of the privately owned beds and leased the publicly owned ones from the State." Holmes' description gives in a comprehensive manner the extent of the oyster ground in the Yaquina River.

During the course of the investigation numerous visits were made to the oyster area and large quantities of oysters were obtained from: (1) the beds of Mr. George Lewis (station 5, Fig. 1), the only privately operated beds which have not come under the control of the Oregon Oyster Company; (2) the planted beds of the Oregon Oyster Company (between stations 6 and 7, Fig. 1 and Fig. 3), containing almost exclusively native Atlantic oysters which were shipped in from the Atlantic coast, and (3) the natural beds (station 4, Fig. 1), leased by the Oregon Oyster Company from the State Fish Commission. However, before these oysters can be discussed more fully, it is necessary to have a general understanding of the life and conditions of existence of oysters, particularly the western species.

Life-History of Oyster

The oyster which is native to the western coast of North America is the small variety, *Ostrea lurida* Carpenter (Fig. 4), and this is the form which is also found to be native on the beds of the Yaquina River. The eastern oyster found on the Atlantic coast is much larger in dimensions and goes under the scientific name of *Ostrea virginica* Gmelin (Fig. 5). This species is not found to

occur in any of our Pacific waters unless it has been introduced through the agency of man.

Our western oyster, the one which is native in the Yaquina River, has taken on distinctive characteristics which mark it off from its eastern relative. While in general many similar factors of food and environment control the life of both of them, yet there are particular differences which mark them off quite distinctly. To a

FIG. 4. Native Yaquina oyster, *Ostrea lurida* Carpenter. Note the young spat near lower margin. $\times \frac{3}{4}$.

very large extent we owe our knowledge of the life-history of our native western oyster to the careful studies of Stafford (1913–1917). Our west coast form is hermaphroditic or bisexual, developing both male and female reproductive organs on the same individual. The east coast form is unisexual, being either male or female and not both. Our western oyster, unlike the eastern one, does not shed its germ cells directly into the water,

where fertilization takes place, but the eggs are fertilized within the oyster itself and here they undergo development for some time before they are discharged into the water. Furthermore, the western oysters reproduce

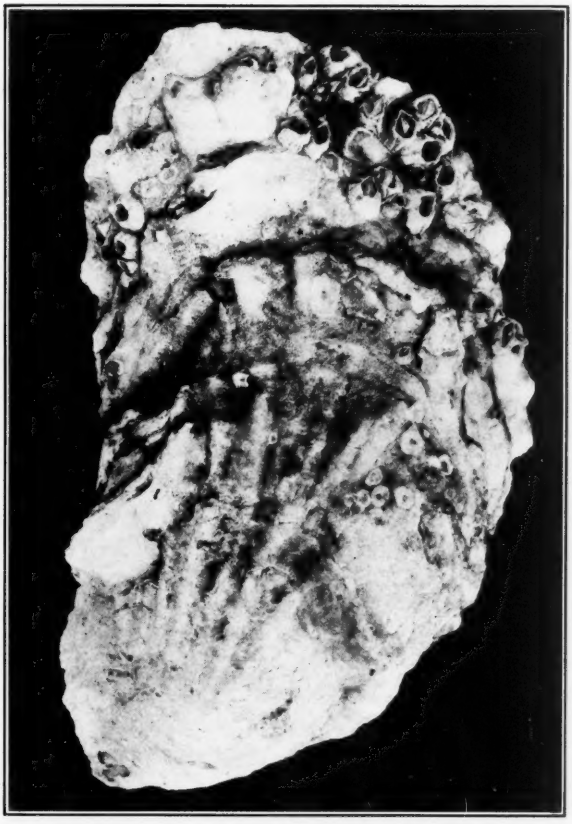

FIG. 5. Transplanted eastern oyster, *Ostrea virginica* Gmelin. $\times \frac{1}{4}$.

young which grow to maturity in our waters. The eastern forms, while they may fatten and grow to enormous sizes and perhaps produce germ cells, do not, as a rule, reproduce young which come to maturity; they are invariably killed off before they proceed very far in devel-

opment. Therefore, from the standpoint of maintaining a constant supply in our western regions, we should be primarily interested in our western oyster, *Ostrea lurida.*

It has been estimated by competent investigators that many of our western oysters attain sexual maturity in one year's time. This period must not be confused with the time required for marketing, for it has been established definitely that it takes about three or four years for our western oyster to attain marketable dimensions. The breeding season comes on in some localities as early as the latter part of May, but does not reach its height until about the middle of June. While the oysters are bisexual, nevertheless the male reproductive organs attain maturity before those of the female. Individual variations take place, of course, some attaining maturity earlier than others, so that while in some forms the male organs are ripe, in others the same organs are past maturity and the female reproductive elements have attained ripeness. The mature male germ cells are discharged into the water and are drawn in by other oysters so that in cases where the female reproductive cells are ripe, they become fertilized and start development into what are known as larvae. The reproductive process thus far described is called "spawning," and according to Stafford (1914) it usually lasts from the latter part of May to the last of July, attaining its peak about the middle of June.

After fertilization the resultant larvae develop inside the oyster for about sixteen days, and then they are discharged into the water, where they swim about freely in swarms for about fourteen days longer. Soon they settle down on some solid object, permanently, as very young oysters ready for development to adult form. The swarming larvae are spoken of as "free-swimming larvae," and when they settle down on solid objects they are called "spat." The objects to which they attach themselves are known as "cultch," and this may consist of

stone, old shells, bits of wood, bark—in fact, any object which affords a solid surface for attachment. The spat, during the first few months of development, are generally alluded to as "seed oysters," and these are the ones which are of great importance in repopulating old, depleted beds or starting new ones. In reality spat may vary in size, from seed to the adult form.

After the seed has once started its progress it must then have suitable care and environment for proper development. If the region is one in which there is not enough food material, the young oysters will starve to death. Also, if they become stationed in a locality where there is much sediment, they will be smothered to death. If, on the other hand, the seed oysters are exposed to drying, or to a lack of salts in the water, they will soon perish. Therefore, it is essential that the oyster culturist familiarize himself with the many points connected with the life and environment of his oysters.

Food of Oyster

In order that oysters may grow normally they must not only find themselves on a suitable bottom, but in a locality which affords them an abundant food supply. The food of oysters consists almost exclusively of minute diatoms, bits of algae, bacteria, Protozoa, and other minute organisms such as crustacean larvae found floating about in the waters over the oyster beds. The main bulk of the food, however, consists of diatoms and plant particles (Fig. 6 and 7). Where stream conditions are favorable these are found in sufficient quantities to maintain the life of the animals. In reality, an examination into the environment in which the oyster lives is a pretty good index as to whether conditions within the stream are suitable for maintaining oysters and other similar animals.

As will be shown later on when the life of the Yaquina is described, the stream contains an abundance of microscopic plants and animals (Fig. 6) floating about within

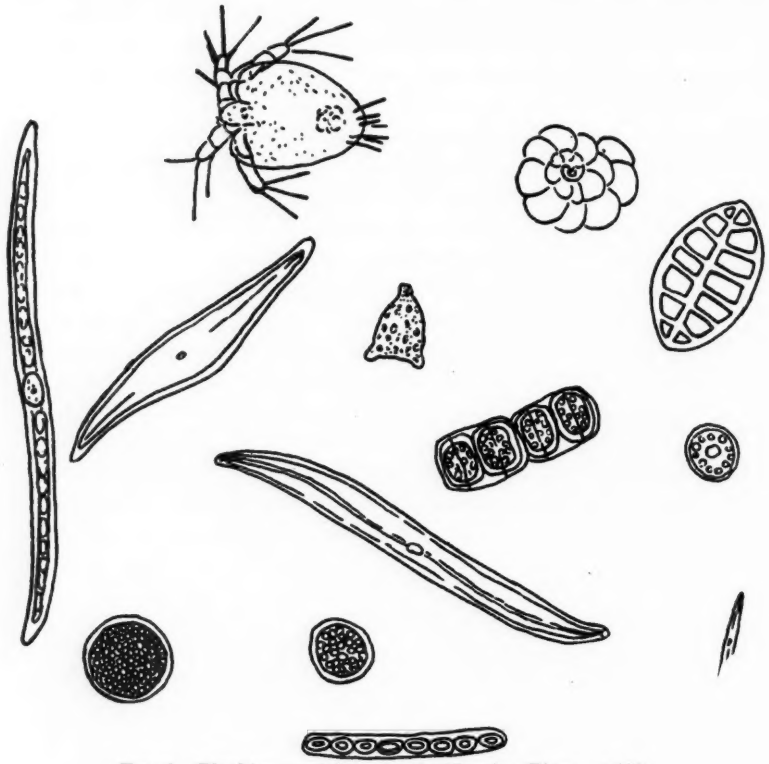

FIG. 6. Plankton organisms in the Yaquina River. ×170.

the water, affording ample food for the oysters. Furthermore, examinations of the contents of the stomachs of many oysters revealed that they are actively feeding on natural food—diatoms, bits of algae and the like—those suspended over the oyster beds. Fig. 7 shows camera lucida drawings of the typical food in the stomachs of Yaquina oysters as revealed by the microscope.

This food is brought into the oyster with water currents that enter the valves. Sand, mud and other particles are also found mixed with the water. The food and bits of organic materials are strained out and then passed to the mouth, where they are taken into the stom-

FIG. 7. Food organisms from stomach of native Yaquina oyster. ×170.

ach. The other solid, inorganic particles are left in the general cavity of the oyster enclosed by the gills, and when the valves open they are quickly removed to the outside by the water currents. Examinations of these solid particles showed that they consisted almost exclusively of sand and mud, similar to the bottom upon which the oyster lives.

SALINITY OF WATER

The salinity of the water was determined by the use of a hydrometer or salinometer which indicated its specific gravity. Ordinarily, the specific gravity of pure water is taken as 1. If there is any salt dissolved, the liquid will be heavier than 1 and the difference between the two fluids shows the amount of salt present. Students of oyster culture have found that waters which vary in specific gravity between 1.008 and 1.024 afford the most desirable localities for oyster beds. In actual practice the best regions have been found to be located between the ranges of 1.012 and 1.020.

In an endeavor to determine the salinity conditions of the waters of the Yaquina River, a number of hydrometer readings were made at various points along the stream on several occasions. The results in each case were approximately similar to those obtained on Satur-

day, October 15, 1927, which are listed below. On this date the determinations were made at ebb tide. By referring to the map, Fig. 1, the stations as given in Table I can be located, at which places specific gravity readings were made.

TABLE I

Station	Place	Specific Gravity
13	Near Toledo	1.004
8	Above Oysterville (Termination of oyster beds)	1.012
7	At Oysterville	1.014
6	In front of Pool's Slough	1.015
5	In Pool's Slough	1.012
4	Below Pool's Slough (Beginning of oyster beds)	1.019
3	At Yaquina	1.022
2	Yaquina Bay	1.024
1	Newport	1.028

The oyster beds, as already mentioned, are situated between stations 4 and 8, and the specific gravity or salinity of the water here varies from 1.012 to 1.019, showing definitely that in this location the water has appropriate amounts of salt for the best development of oysters.

LIFE IN YAQUINA RIVER

Just as the barometer indicates the climatic conditions of the environment, so also does the life in a stream indicate whether conditions are wholesome within it. In attempting to ascertain the conditions which surround the oyster beds, particular attention was paid to the life which exists at the present time within the river. The assumption which guided this investigation was that if the stream possessed a normal and healthy life, then the probability is that conditions are normal. On the other hand, if life is scarce, then one would be justified in concluding that conditions are unfavorable within the stream.

That portion of the stream between Toledo and Oysterville was most carefully investigated, and here the life was found to be abundant, vigorous and perfectly normal. The surface catches of plankton as revealed through microscopic studies of the contents showed large numbers of diatoms, algae, Protozoa, minute Crustacea, particularly copepods, and numerous larvae of such crustacean forms as crabs, barnacles, copepods and the like.

The bottom and shore showed a large variety of different organisms. There were numerous Crustacea such as free-living crabs, hermit crabs, barnacles and beach fleas (amphipods and isopods). Buried in the mud were found numerous sand and tube worms. Molluscs also abounded here, there being sea-snails (*Thais*), sessile barnacles, mussels, clams, cockles, oysters, and jelly-like egg masses of snails deposited on various sea weeds. Fig. 8 shows some of the common species of molluscs. Many of the molluscs had sea weeds and bryozoan sea mats covering their surfaces. Here also were found numerous sea anemones, hydroids and sponges. When a microscopic examination was made of the muddy bottom of the river, an abundance and variety of minute forms, such as Protozoa, diatoms and fragments of algae could be distinguished.

At the time when the investigation was being conducted large numbers of salmon of various kinds were running up the river, and fishermen all agreed that it had been one of the most prosperous seasons for salmon fishing that they had known in years. Other fish were also found in the stream, but little attention was paid to them, inasmuch as interest was centered chiefly in those forms that are commonly associated with normal and healthy conditions that surround oysters. The oyster beds in particular showed an abundance of the life which has been mentioned, indicating quite clearly that conditions under which the oysters exist are both normal and natural. If such were not the case, then the life of the stream, particularly the microscopic life, would be depleted and extinct!

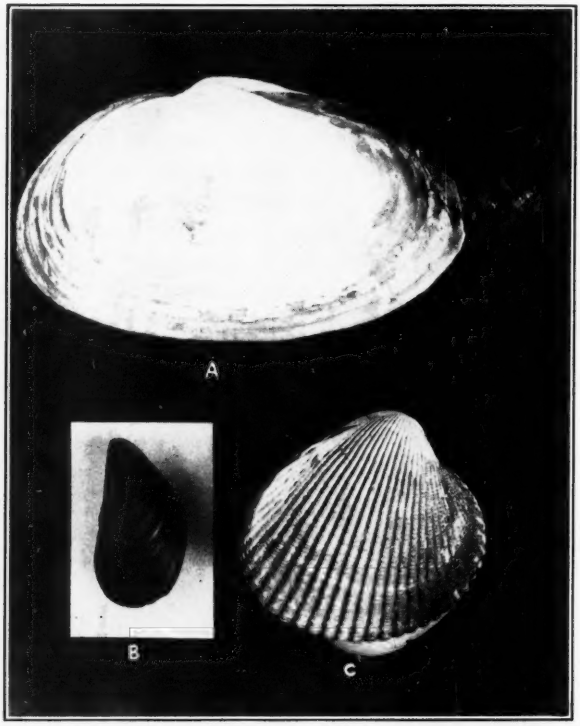

FIG. 8. Common species of molluses found in Yaquina River. A, *Mya arenaria;* B, *Mytilus edulis;* C, *Cardium corbis.* × ½.

HISTORY OF OYSTER BEDS

As far as could be ascertained, the natural oyster beds in the Yaquina have existed from time immemorial. Oystermen of the district who have lived in the region for a long time are of the opinion that the first white man to discover the oyster beds was the father of Mr. George Lewis, a fisherman who now resides at Oysterville, having been born there about sixty years ago. Even Mr. George Lewis himself can not give the exact date of their discovery. Conservatively estimated, the beds have been worked by the white man at least seventy years, and during this time tremendous quantities of

oysters have been brought up and utilized locally or shipped to distant markets such as Portland and San Francisco. There have really been three distinct periods in the history of the oyster beds of the Yaquina region.

1. *Early Period.* This period, running from about 1860 to 1870, involves the time when the oyster beds were first discovered by the white man, when population in the west was scarce and the only large settlement was that of San Francisco. The old-timers of the Yaquina state that during this period large numbers of schooners came up the Yaquina River and dredged out tremendous quantities of oysters, virtually taking them out by scow loads, and transporting them by boat to the San Francisco markets for consumption. No thought was given at this time to conservation, for no one dreamed that the oyster beds could ever be exhausted.

2. *Middle Period.* This refers to the period when the state of Oregon and private citizens of the state definitely obtained control over the oyster beds. It runs from about 1870 to 1923, and during this time the beds were worked heavily and continuously, and no thought was given to replenishing the diminishing supply. Many of the beds became so depleted that they were virtually exhausted. During the last few years of private control the oystermen encountered considerable difficulty and catches were materially decreased. The important fact to bear in mind is that no attempt was made towards experimentation for the purpose of maintaining or increasing the yield of oysters.

3. *Recent Period.* This dates from the year 1923, when the Oregon Oyster Company took over most of the private beds and leased the state's natural beds. When this concern got control of them they were already in a dangerous state of depletion. Instead of surveying them carefully for purposes of applying measures which would build them up and conserve the fast diminishing supply of oysters, they rather increased the damage by their heavy dredging and tonging operations. Many of the

beds which were in a state of partial depletion were practically wiped out by such methods, and needless to state, this has proven disastrous to the industry.

In going over the history of the oyster beds, it becomes apparent that they have been in a state of depletion for a good many years. The following quotations from official bulletins published by the state of Oregon are extremely significant. In the first biennial report of the state biologist for 1901, Appendix C—"Present Condition of Eastern Oyster Experiment and the Native Oyster Industry," F. L. Washburn, state biologist, says, "Yaquina Bay oystermen get at present $2.50 to $2.75 per sack for native oysters, a San Francisco firm having contracted with most of the oystermen for this season's output at the latter figure. The oysters on the native beds are so closely worked now that one-half a sack on a tide is considered fair work, though one sack is sometimes obtained. In the past, the business has been much more profitable than at present. The oysters have dwindled in numbers and in size, owing to a too persistent tonging, together with a lack of foresight on the part of the oystermen. If they could unite in a determination to forbid all tonging for two years or more on certain reserved portions of the natural beds, and persist for a number of years in such a plan, using care with the unmarketable seed, besides taking the best possible precautions along modern lines for catching spat, I believe the industry could be restored to something like what it was ten years ago. But if the present methods continue, I will predict the extinction of the industry before many years."

Two years earlier, in 1899, this same investigator, in an article in *Popular Science Monthly* entitled "Eastern Oyster Culture in Oregon," made somewhat similar significant assertions. He said, "*Ostrea lurida,* the toothsome little native oyster which years ago was so abundant at Yaquina Bay, affording support to many families, has decreased in numbers to such an alarming extent that unless some radical measures are soon taken to prevent,

the native oyster industry of this locality will be a thing of the past."

In the second biennial report of the state biologist under date of 1905, Albert R. Sweetser, then state biologist, in speaking of the eastern oyster experiments in the Yaquina region urges the need for such research, especially for the purpose of obtaining "a species that will multiply and be available to eke out the insufficient supply of natives."

In the fourth biennial report of the state biologist dated 1909, State Biologist Albert R. Sweetser, in his letter of transmittal, makes the following comment on the diminishing yield of oysters. "The experiments with the eastern oysters have shown that it is impossible to make them breed in these waters because of the low temperature, and attention is being turned to native oysters and the possibility of increasing the supply on the natural beds or stocking new localities, the latter being of special interest in Coos Bay, where at present the oyster is extinct though at one time flourishing abundantly. It seems very desirable that the appropriation should be continued that part of it may be used in such experiments." In the same report he also states, "These results suggest other problems. 1, The feasibility of raising spat on a larger scale by some similar method and restocking the beds which are rapidly being depleted."

In December, 1918, Carl D. Shoemaker, then state game warden for Oregon, made the following statements in his 1917–1918 Report to the Board of Fish and Game Commissioners: "The natural restocking of the oyster beds of Oregon has fallen off to such an extent that the industry to-day can hardly be said to be profitable. Yaquina Bay offers the most favorable location for artificial restocking of this excellent food. I would recommend that legislation be enacted giving greater latitude to the Fish and Game Commission, to the end that this work may be better carried on."

In going over the beds and in talking with the oystermen it becomes apparent that practically nothing has

been done in bygone years to conserve the oysters for future generations. The oystermen have been interested exclusively in exploitation for gain, and have done almost nothing to help nature along in replenishing the supply. It must be remembered that the beds of the Yaquina region have been extremely limited. For instance, in the Third and Fourth Annual Reports (1895–96) of the State Fish and Game Protector, Hollister D. McGuire, to Wm. P. Lord, Governor of Oregon, we meet with the following assertion: "The oyster industry of the state at the present time is of small importance, judging from amount and value of the output. The industry of Oregon is confined to Yaquina Bay, at which place there are twelve private oyster beds, staked as provided by law; also, a small area of natural beds from which the supply for renewing the private beds of the oystermen is obtained. The output of the oyster beds at Yaquina Bay from June 15, 1895, to June 15, 1896, amounted to two thousand sacks of one hundred pounds each, the most of which was shipped to the San Francisco market and brought the oystermen $3 per sack, or a total of $6,000 for the year." Then again, in the Fifth and Sixth Annual Reports (1897–98) of the State Fish and Game Protector, Hollister D. McGuire, to Governor Wm. P. Lord, of Oregon, the following pertinent statements are made: "The oyster industry of the state is at present confined to Yaquina Bay, and is of small proportions. The native oyster of this bay is noted for its excellent flavor and brings a higher price than the native oysters of Puget Sound and Grays Harbor. From June 15, 1896, to June 15, 1897, there were shipped from Yaquina and consumed locally 1,406 sacks of oysters, which brought the oystermen $3 per sack, or a total of $4,218, most of the output going to San Francisco."

It is thus evident that the oyster area has been quite limited and therefore only a limited supply of oysters could be expected annually. Obtaining more than this yield soon upsets the natural balance with the result that

fewer and fewer oysters remain on the beds and so less and less young are annually produced. It is perfectly obvious that such a procedure soon wipes out any natural resource, and this is exactly what has been happening to the oyster beds of the Yaquina region! Conditions would not have been quite so bad if the oystermen had made an intelligent attempt to study the situation for the purpose of finding a remedy which would counterbalance the depletion; but as far as could be determined, almost no scientific work of such a character has been undertaken.

Prior to 1923, the date when the recent period in the history of the oyster beds began, the oystermen operated in a limited way, and in spite of the fact that the beds were already in a state of depletion and also that the oystermen had difficulties such as were pointed out previously by Edmondson, they made a fair catch which netted them a fair return. Since 1923, there has been no let-up and the exploitation of the oyster beds has increased to the extent where many of them have become seriously depleted. In order to increase yields, many of the adult oysters with spat were dredged and tonged up from the natural beds and then distributed either on locations which were devoid of oysters, or into the main channel of the river where there were plantings of eastern oysters from the Atlantic seaboard. Such operations soon prove fatal to oyster beds, for the following reasons. In the first place, it removes large quantities of adult oysters and young growing spat from locations where they are developing in a healthy manner. Secondly, transferring them on to depleted areas in the main channel of the stream is decidedly bad, for the oysters are soon covered with mud and silt to an extent where they are virtually buried. The result is that many of them are either killed off or their normal growth is greatly interfered with. Finally, when mud and silt cover the shells they no longer serve as cultch, for this débris makes it impossible for the free-swimming larvae to come in contact with the clean surfaces of the shells

in order to affix themselves. These findings are in agreement with those of Moore (1897), Stafford (1913, 1915), Churchill (1920) and others who have pointed out repeatedly the necessity of favorable bottom locations for successful oyster culture.

Aside from interfering with the natural oyster beds and lack of proper planting of oysters on suitable bottoms, almost no work has been done by the oystermen in supplying the beds with proper cultch at the right time so that swarming larvae will have an opportunity to attach themselves. As already mentioned, cultch refers to the objects to which larval oysters attach themselves. This may consist of pieces of wood, stones, shells of various molluscs and objects of similar character. It has been found that clean shells of oysters or clams, which have been allowed to remain in the open where they can be washed by the rain and bleached by the sun, afford the cheapest and best means of cultch. At the appropriate time, shortly before the larvae of the oysters are ready to attach themselves, these shells must be distributed over the oyster beds in order that the young oysters have an opportunity of attaching themselves. The best time for this depends on microscopic studies of the organisms in the waters over the oyster beds. When large numbers of oyster larvae make their appearance in the catches then is the best time to distribute the cultch!

Stafford in his work with the native oyster of British Columbia, as reported in the publications of the British Columbia Fisheries Department for 1917, tells us that "The time to put out cultch has been and still is largely judged by the results of previous plantings, *i.e.*, by experience. A sort of customary time—about the last of June or first of July—has been arrived at. But this is not equally good for all places. It may be a little late for places to the south or somewhat early for those of the north. Then again, it is not equally good for all years, for the warm weather of one summer may be con-

siderably earlier than for another. There are other things that may interfere, such as sudden changes of temperature or a heavy fall of rain. It is useful to have in mind some approximately correct time as a reminder that certain preparations should be commenced, but the actual time can not be foretold with accuracy for any considerable period in advance. It has to be determined for each year, and, in fact, for each locality, except where places are near together and under like conditions."

In the Yaquina region very little attention has been paid to the problem of cultch. While some shells have been scattered over the beds in a hit or miss manner no systematic attempts have been made in this direction year in and year out. The oysterman has relied largely on nature to do his work, and as already mentioned, nature has limitations beyond which she refuses to go. Man must then come in and afford help or improvement if he wishes to prevent exhaustion and final extermination. In studying the oyster beds, it soon becomes apparent that the oystermen on the Yaquina have done very little in the past to build up the beds or to conserve the supply of oysters on them. This has been the situation for a good many years, with the result that the oyster beds are greatly depleted and the yield from them has become accordingly diminished. With adequate study, care and the application of stringent measures of conservation many of these areas may be revived and the yield increased accordingly, but this will have to come through careful scientific study and research.

Present Condition of Oyster Beds

Numerous visits were made to the oyster beds to determine their condition. On October 2nd and 16th Mr. George Lewis conducted me over his oyster beds at Pool's Slough, located in the heart of the oyster district at Oysterville (station 5, Fig. 1 and Fig. 9). Mr. Lewis and I tonged up large numbers of native Yaquina oysters and these were turned over to me for examination. The

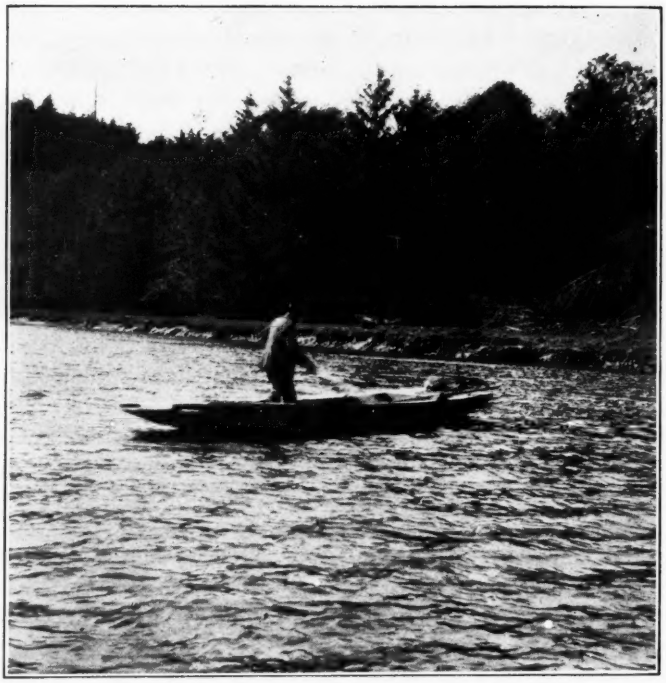

FIG. 9. Oystering in Pool's Slough

oysters were attached to shells of clams, cockles, native and eastern oysters. There were an exceedingly large number of dead shells among them. The majority of the live oysters obtained were two years old, and these were in excellent condition, fat and healthy in appearance. Many were opened on the ground and the mantle cavity was carefully examined. Nothing but sand, mud and fragments of sea weeds were found. They contained exactly the same material as the bottom upon which they were living. Harlan B. Holmes, of the U. S. Bureau of Fisheries, reports similar findings. He states that "in examining the oysters special attention was given to the presence of foreign matter in the mantle cavity. Nothing of any significance was found and the

oysters appeared to be in a normal and healthy condition."

A few cockles and clams were also obtained and these were in fine condition. Included in this catch were also a few specimens of the sea snail (*Thais*), hermit crabs and small Oregon crabs. Some of the oysters had sponges, sea anemones, hydroids, sea mats (bryozoa), sea mosses and algae growing on them. Microscopic examinations of their surfaces later revealed great numbers of diatoms, the natural food which the oysters use. Everything indicated that the bottom of the oyster beds was perfectly normal and in good condition for the existence of living organisms, such as oysters and those forms ordinarily associated with them.

Samples of this bottom débris were bottled and taken to the laboratory for examination, and these were found to consist of sand and mud, rich in diatoms, algal particles, plant fragments, Protozoa and larvae of Crustacea. The diatoms predominated by far. Many of the oysters were also taken to the laboratory for microscopic examination of stomach contents and their food was found to consist of organisms (Fig. 7) similar to those existing in the bottom samples above mentioned.

In spite of all this the contents brought up showed that the oystermen had not given much care to the oyster beds. Spat was in evidence, but this was clustered and crowded on the shells. The oystermen had not gone over the beds for purposes of separating the young oysters so that they would have the opportunity of growing more effectively. Furthermore, the spat seemed to suffer from a lack of good cultch. While shells were present they appeared to have been on the beds a very long time and were covered with large numbers of barnacles, débris or slime, which prevented many spat from attaching themselves. It seemed evident that no systematic efforts had been made by the oystermen to build up or maintain the beds by the logical accumulation of cultch and its distribution over the beds at the proper time.

On other occasions large numbers of oysters were obtained off the natural and planted beds controlled by the Oregon Oyster Company. From the natural beds in the main channel and near the west and southwest shores in the vicinity of station 4, Fig. 1, about two bushels of native oysters were obtained and carefully examined. Many of them were opened directly on the ground and were observed to be in a healthy and normal state. The beds upon which they were located revealed nothing but mud and sand. Bottom dredging in this vicinity brought up only mud and sand. Under the microscope this material was found to be rich in normal oyster food. Laboratory examinations of the stomach contents of the oysters disclosed the typical oyster diet, consisting largely of diatoms and algal fragments. Also, oyster spat of various ages was in evidence here (Fig. 10).

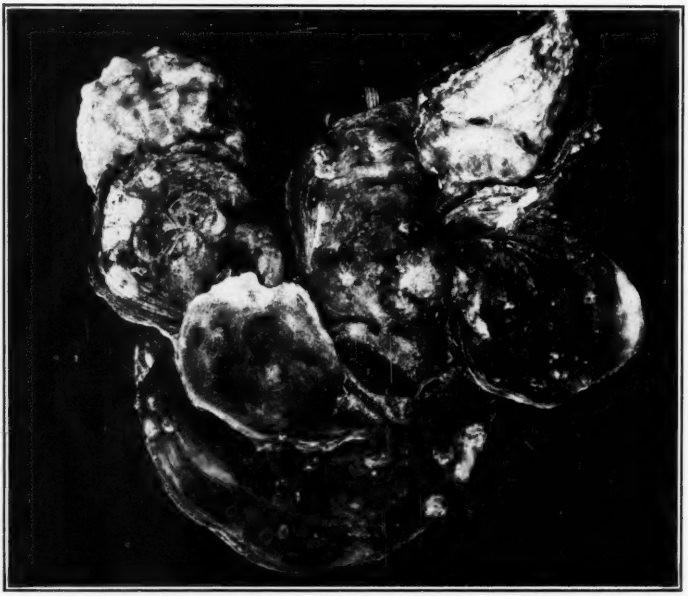

FIG. 10. Spat of native Yaquina oysters. ×¾.

In spite of all this, it soon became evident that the beds were in very poor condition, like those in Pool's

Slough. When I remarked about the condition of these oyster beds to Messrs. George Lewis and Tom Christensen, fishermen residing at Oysterville, they responded by saying, "Why shouldn't the beds be in poor shape? They have been neglected and given no care. Furthermore, in recent years they were stripped of most of the oysters by heavy tonging or dredging operations and this has proved ruinous to them."

From the planted beds of the Oregon Oyster Company many eastern oysters were obtained through Mr. Meinert Wachsmuth, one of the owners of the firm residing at Oysterville. The beds where the eastern oysters were planted lie on the shore flats below low water mark, starting a little below Mr. Wachsmuth's home and running half way the distance towards Pool's Slough. The location is along the south shore in the vicinity of station 7, Fig. 1. Fig. 3 indicates the location. It is of interest to note that the beds upon which the eastern oysters were planted at one time were rich in native Yaquina oysters, but because of heavy oystering they have been wiped out almost entirely, so that at present only a very few of the native forms are ever obtained on them.

The eastern oysters which were planted were adults, about three or four years old, with the idea undoubtedly in mind of having them available for market very soon after planting. The Oregon Oyster Company did not bother with seed oysters, which are generally around a year old, because these would have to feed and grow for about three years before they would reach marketable sizes. It is important to bear these facts in mind in order to explain the mortality which has been going on amongst the transplanted adults.

Mr. Wachsmuth brought up over five bushels from his oyster beds, consisting almost exclusively of adult eastern oysters, either alive or dead. Included in this catch were large quantities of black mud, silt and sand, samples of which were obtained for examination. Dredgings in the main channel near these beds gave similar

bottom samples. In the laboratory these materials showed the usual oyster food, such as has already been described. Another interesting feature of this catch from the planted beds was the fact that over half of it consisted of dead eastern oyster shells. The oysters which were alive consisted of a few native Yaquinas which were in excellent shape, and the rest, *i.e.*, the vast majority of them, eastern oysters. These latter could be classified into two groups: (1) healthy, grayish and fat, and (2) sickly, greenish and thin. An examination of both these latter groups, particularly of the second, showed that a large number of the shells had been attacked by the boring sponge found on many of the oyster beds of the Atlantic coast. These shells were riddled with numerous holes and excavations which weakened and destroyed them. The sponge, in its boring operations completely perforates the shell so that the oyster is constantly using up its energy in healing over these places. The result is that the oysters are weakened to such an extent that many of them die. In the burrows of the shells, immediately underneath the outside layers, there were invariably found annelids and other minute organisms, depriving the oysters of food and adding to the damage, thereby hastening their death. Fig. 11 is a photograph of one of the transplanted adult eastern oysters from these beds, showing the condition.

On the shore beside Mr. Wachsmuth's home there was a large pile of eastern oyster shells and when these were examined it was found that the shells showed distinct traces of having had live oysters in them and opened recently. Furthermore, nearly half of the pile consisted of shells which were riddled by the excavations caused by the boring sponge. Undoubtedly this disease was brought with the oysters from the east, for, as has been pointed out, many of the areas on the Atlantic coast have suffered from the ailment. That this is not a disease of the Yaquina oysters is borne out by the fact that in not a single instance was it discovered on any of the native oyster shells examined.

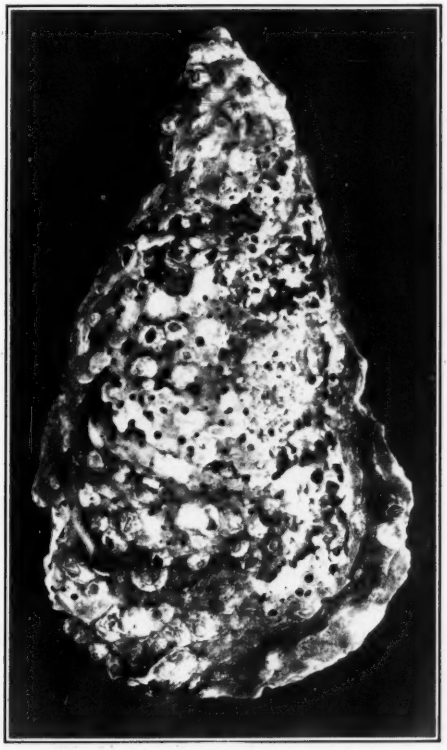

FIG. 11. Eastern oyster showing effects of boring sponge. Young spat of native oyster near lower margin. $\times \frac{3}{4}$.

Many of the live oysters were opened and examined on the ground. Careful search of the mantle and gill cavity revealed sand and mud particles, similar to the bottom on which the oysters were living. About a bushel of the eastern oysters and many of the native Yaquinas were taken to the laboratory for study. Examinations under the microscope of stomach contents revealed natural oyster food—diatoms, algae, Protozoa and the like similar to the organisms dwelling on the surface and bottom areas where the oysters were living.

While spat was scarce, nevertheless it was found on quite a number of shells (Fig. 12), even settling on shells

FIG. 12. Eastern oyster with spat of native Yaquina oysters. $\times \frac{3}{4}$.

which were riddled with cavities produced by the boring sponge. Of course, this spat consisted of the native Yaquina oyster, *Ostrea lurida,* and not of the transplanted eastern oyster, *Ostrea virginica,* which apparently does not reproduce in the Yaquina location.

Concerning the condition of the eastern oysters, there are numerous reasons which account for it.

(1) From all the information available, the oysters, when they arrived from the east, were in exceedingly poor shape. Fishermen in the vicinity stated that about half of the oysters shipped from the east arrived either dead or in a dying condition. Many of the live ones had

their beaks broken off, exposing the soft inner parts, making it rather easy for enemies to enter and destroy them.

(2) Large numbers of oysters shipped were undoubtedly parasitized by the boring sponge so that this disease was transplanted to the Yaquina from the eastern locality where the oysters developed. Inasmuch as this parasite weakens, thins and ultimately kills oysters, there is not a question of a doubt that many of the oysters which were planted soon died on their new location in the Yaquina River.

(3) The eastern oysters which were planted were adults, about three or four years old. In practice it has been found that the best results are obtained by planting seed oysters, those which are small and around one year old. The logic of this is quite obvious. The adult oysters had accustomed themselves to a certain environment on the Atlantic coast, and then they were shipped nearly 3,000 miles to their Pacific location on the Yaquina River, where they had to meet different conditions and acclimate themselves to a virtually new environment. Young oysters are quite plastic and can adjust themselves very readily to such changes. On the other hand, the adults have become more or less fixed and are not able to orient themselves nearly as readily. The same parallel may be drawn between the adjustments of children and adults among humans. In spite of all this, some of the eastern oysters did adapt themselves to their new locations and have grown into fat and healthy individuals. Many others, however, could not so adjust themselves and soon died. A large number of those which were weakened in transit or arrived in a diseased state also succumbed. The sum total of all this has been that many dead oysters are found at the present time on the planted beds.

That the adult eastern oysters seem to succumb more readily in the Yaquina region than the young ones is a matter of official record. The first shipments of eastern oysters to the Yaquina region came from the U. S. Bureau of Fisheries between the years 1896 and 1899,

and they consisted of 32 barrels of seed oysters. They were planted near Oysterville and for a few years seemed to be doing exceptionally well. However, in 1904, State Biologist Albert R. Sweetser, in his second biennial report, tells us in reviewing eastern oyster experiments that "some of the oysters have reached the age of six or seven years, which some regard as the limit of life, though others maintain that they can continue to grow and fatten for a number of years more. We are finding many empty shells and it would seem that they are dying of old age."

Then again, in the third biennial report for 1907, State Biologist Albert R. Sweetser, in commenting on the eastern oyster experiments, remarks, "It was decided to transfer the oysters to new beds in search of more favorable environment, and also to protect them from the injury by the dragnets of the fishermen. . . . They were situated on the north shore of Yaquina Bay near the railroad station of Winant. Mr. George King offered the use of one of his private beds on the south side and almost opposite. . . . In making the transfer many empty shells and dead oysters were found."

(4) The eastern oysters were planted in a bad region, which received large quantities of silt, mud and sand, burying the oysters, killing many of them off and preventing spat from settling on their shells. The question of where the best place is for planting the eastern oysters in the Yaquina River has never, to my knowledge, been accurately determined. As has been pointed out, the area in which they were planted does not seem to be the best section. It appears that no observations or experiments have been made to determine the locations most suitable for obtaining the best results with eastern oysters. The procedure has been largely a hit or miss proposition, and unquestionably this has accounted for the results obtained.

That eastern oysters will fatten and grow large in the Yaquina River is evident from the fact that quite a number of the transplanted forms have established them-

selves and have grown into vigorous and healthy individuals (Figs. 5 and 12). With careful study of the environment there is no reason why it could not be determined where the raising of these forms could be accomplished with greatest success.

However, it seems fairly certain that these eastern oysters do not produce spat that will perpetuate the species. Numerous experiments have been tried in the early days to induce eastern oysters to become established and reproduce themselves in the Yaquina, but ultimately they were all given up as failures. In 1908, State Biologist Albert R. Sweetser, in the letter of transmittal which accompanied his fourth biennial report to Governor Chamberlain, wrote, "The experiments with the eastern oysters have shown that it is impossible to make them breed in these waters because of the low temperature." A similar state of affairs has been found to exist in practically all other oyster areas along the Pacific coast.

Concerning the question as to why large numbers of spat of the native Yaquina oysters are not found on the shells of the transplanted eastern ones, the reasons unquestionably are as follows: In the first place, the eastern oysters were planted on a bed where almost no native oysters remained. The latter were tonged or dredged up in bygone years without allowing the beds to repopulate themselves. Therefore, at the present time, there are hardly any of the breeding adults left in the region and therefore very few larvae, if any, are available for setting. In the second place, the eastern oysters were in a location where large quantities of mud and silt cover them, with the result that larvae in this region have found it difficult to encounter clean surfaces of shells to which to attach themselves. As has already been mentioned previously, spat has been encountered on quite a number of the eastern oysters indicating clearly that if clean shells were placed in the water at the right time, in regions where native Yaquina oyster larvae abound, a good crop would undoubtedly result.

Finally, after a careful consideration of all of the evidence the chief reason for the depletion of the Yaquina oyster beds, aside from the natural obstacles mentioned above and the limited area, has been the devastating practice of exploitation which has been going on, without adequate and scientific measures of conservation to maintain the supply. This question has already been alluded to and it will be necessary to repeat only the salient facts. The oyster beds have been neglected; many natural beds have been ruined by dredging and tonging operations; the best regions for spat, such as sloughs, have not been utilized or developed; no scientific work has been done to determine time of spawning; no adequate provisions have been made for cultch; no attempts have been made to overcome natural obstacles presented by the Yaquina River, such as mud, silt and sand distributed by the tides. In a word, the greatest enemy to the oyster beds of the Yaquina region has been the oysterman and his unscientific methods of exploitation without adequate measures of conservation. As will be recalled, State Biologist Washburn sounded the alarm with regard to the Yaquina oyster beds as far back as thirty-two years ago!

In recent years, the oystermen instead of attempting to improve conditions have simply added to the ruin by their unscientific methods and practices. After all is said and done, it must not be forgotten that where a limited producing region is available, only a limited natural yield is possible. Stretching it beyond this point is soon disastrous, unless intelligent care and adequate conservation measures are applied to maintain it. Even then, nature often refuses to bring forth the abundance expected, with the result that exhaustion or extinction invariably follows. This is what has happened to most of the oyster producing regions on the Yaquina River!

SUMMARY

1. The Yaquina oyster beds of Oregon are located in that portion of the Yaquina River lying mainly in the

vicinity of Oysterville, beginning approximately six miles below the town of Toledo and extending for nearly three miles in the direction towards Newport.

2. The oyster beds are limited in area, comprising no more than 135 acres at the most. The large bulk of the oysters consists of the native Pacific species, *Ostrea lurida* Carpenter. There have also been numerous plantings of the Atlantic oyster, *Ostrea virginica* Gmelin, but with few exceptions these have not acclimated themselves to their new locations in the Yaquina region.

3. The Yaquina River is an exceedingly narrow and shallow stream carrying a large amount of sediment and débris. Inasmuch as this suspended material settles on the oyster beds, it becomes absolutely necessary for the oystermen to keep constant watch over their beds and give them the very best of care and cultivation.

4. The salinity and food in the vicinity of the oyster beds are adequate for the proper development of oysters. Moreover, there is an abundance of natural life in the Yaquina River, indicating that stream conditions are natural and wholesome not only for oysters, but also for those forms invariably associated with them.

5. Untreated human sewage enters the Yaquina River from the various towns located along its banks and this is then carried by the tides back and forth over the oyster beds, making it possible for the oysters to become polluted with dangerous disease producing organisms.

6. The oyster beds have been steadily and consistently worked for the last seventy years. A large quantity of oysters has been removed and little attention has been paid to conservation measures looking towards the building up and maintenance of the beds. The inevitable result has been that many of the beds have become exhausted and the yield of oysters has diminished to the extent where the remaining beds are threatened with extinction.

7. Carefully planned surveys and experiments will have to be initiated to maintain, replenish and repopu-

late the Yaquina oyster beds if they are to be conserved for future generations.

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SHORTER ARTICLES AND DISCUSSION

ORDER OF EMBRYONIC SEGREGATION IN INTERMEDIATE APHIDS NOT REVERSED BY LOW TEMPERATURE[1]

In a study of the composition of intermediates between gamic and parthenogenetic aphids (Shull 1930b) it was found that a change from parthenogenetic to gamic individuals produced the same type of intermediates as did change from gamic to parthenogenetic, and that the order in which the distinguishing marks became altered was reversed in the two directions of change. When the offspring were changed from gamic to parthenogenetic, by removing their winged parents from low to high temperature, the earliest offspring to show an effect of this change possessed modified antennae and tibiae, and only in offspring born later were the reproductive system and the color of the body altered. In these modifications of the reproductive system, the earliest offspring lost their colleterial glands and seminal receptacle, while later ones exhibited various changes in the ovarioles. The order of change in successive offspring is thus antennae and tibiae, then colleterial glands and seminal receptacle, then ovarioles, with body color changing along with the latter two changes. When the offspring were changed from parthenogenetic to gamic, by changing the winged parents from high to low temperature, the earliest intermediate offspring showed modifications of the reproductive system and body color, while only later offspring showed changes in the antennae and tibiae. The intermediate reproductive systems were too few in this latter transition to fix certainly the relative time of change of colleterial glands and ovarioles, but aside from this the order of change in successive offspring was the reverse of the order of change when gamic offspring were being gradually converted into parthenogenetic.

The nature of these intermediates is of considerable genetic interest partly because of the probability that they are produced under the same fundamental conditions as are intersexes. Goldschmidt explains intersexes as due to a dichotomy of embryonic

[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

segregation (embryonic determination) plus a turning point in physiological properties affecting development. The dichotomy of segregation entails the determination of different structures successively instead of simultaneously, a condition well established in a number of vertebrate animals. The turning point, so far as concerns intersexes, is the time at which, owing to a rise in the level of certain processes, developmental influences change from female-determining to male-determining or the reverse. Structures segregated (determined) before the turning point are like those of one sex, those segregated after the turning point are like those of the other sex. Goldschmidt has summarized his theory of intersexes in a recent review (1931) in which his earlier papers and other pertinent literature are cited.

If Goldschmidt's theory is applicable to intermediate aphids, those intermediates which are produced during a change from parthenogenetic to gamic should possess different characters from those produced during a change from gamic to parthenogenetic. The order in which the structures change should be the same in both, but since in one of the changes a given organ should appear while in the opposite change that organ should be lost, the composition of the intermediates produced should be quite different in the two cases. As stated above, these conditions did not exist in the intermediates obtained in my earlier work; the order of change was apparently reversed, while the composition of the intermediates was the same regardless of the direction of change.

Three possible explanations of this result were discussed but none of them adopted in preference to the others for want of decisive evidence. Two of them permitted the adoption of Goldschmidt's theory, the third was opposed to it. These explanations were briefly as follows: (1) that the intermediates which were born during the transition from parthenogenetic to gamic were not produced then but had been produced earlier during a change from gamic to parthenogenetic (it had been impossible to get a transition from parthenogenetic to gamic except in families that had first been changed from gamic to parthenogenetic by high temperature), and were necessarily of the same composition; (2) that the low temperature which induced the change from parthenogenetic to gamic offspring had, by a differential effect on the rates of development of different structures, reversed the order of segregation of antennae and

tibiae as compared with the reproductive system; and (3) that the nature of intermediates is due, not to the order in which their structures are embryonically segregated, but to the level at which some physiological property exists regardless of the direction from which that level has been approached. The first of these explanations permits the adoption of Goldschmidt's theory but does not necessarily favor it; the second directly recognizes that theory and is built upon it; the third is opposed to Goldschmidt's theory.

Some evidence has now been obtained which tends to show that the second of these explanations is incorrect. It consists of a number of winged aphids, of the same species as was previously used (*Macrosiphum solanifolii*), which possessed thickened hind tibiae. Gamic females, which normally possess thickened hind tibiae, are regularly wingless. Winged individuals with thickened tibiae are therefore one form of gamic-parthenogenetic intermediate. Two females of this kind had been previously obtained, several years apart, and their significance appreciated; but they were too few to form the basis of any valid conclusion. Now, however, 34 more of this kind have been produced, in a single experiment in which several hundred intermediates of various types appeared. Within this single experiment it would seem to be particularly safe to compare the intermediates with one another.

While all of these intermediates are being used in a comprehensive study of intermediates in general, it is with respect to only one feature of them that the present paper deals, namely, the relative thickness of the hind tibiae in winged and wingless intermediates. The tibiae of the winged intermediates are thinner than those of the wingless ones. The method of recording thickness of the hind tibiae, as described in an earlier paper (Shull 1930a), is to rate the thick tibiae of the gamic females at 0, the thin tibiae of the parthenogenetic females at 4, and the intermediates at regular intervals (1, 2, 3) between these extremes. A comparison is here made only between intermediate females appearing in the same experiment. Since all the winged females with somewhat thick hind tibiae had reproductive systems that also were partly parthenogenetic and partly gamic, they are compared only with wingless females whose reproductive systems are in some manner a mixture of the two types. In this comparison it is found that the mean thickness of the

hind tibiae of the winged gamic-parthenogenetic intermediates was 3.14 ± .07, while that of the wingless gamic-parthenogenetic intermediates was .74 ± .09. A more restricted comparison may be made by selecting only those possessing the same type of intermediate reproductive system. Of the winged ones all but two had parthenogenetic ovarioles but colleterial glands and seminal receptacles. The 32 possessing such reproductive systems had hind tibiae averaging 3.10 ± .07, while the 29 wingless intermediates with the same type of reproductive system had hind tibae whose thickness was .82 ± .15.

It is clear from the above that possession of wings in a gamic-parthenogenetic intermediate carries with it a thinning of the hind tibiae. Without implying that any such physiological relation is involved, it is as if something was consumed in producing wings which would otherwise have gone into thickened tibiae. Now, it was shown in earlier experiments (Shull 1928) that whether a female is to be winged or not is not decided (at least so far as light and darkness are concerned) until the last 48 hours (approximately) before birth, or probably even within the last 24 hours. Presumably, therefore, from the correlation described above, the thickness of the tibiae is likewise not determined until shortly before birth. Since it has been found impossible to alter any of the aphids by applying to them after birth the treatments that have been shown to be efficacious before birth, it appears almost certain that the wings and the nature of the hind tibiae are among the last things to be segregated in the course of embryonic development.

What does this fact signify with regard to the three possible explanations described above as accounting for the similarity of intermediates produced during changes in opposite directions? All of the winged gamic-parthenogenetic females described in this paper were the offspring of parents reared at low temperature until just before these intermediates were born. If low temperature has a differential effect on the rate of development of tibiae as compared with reproductive systems, such as to reverse the order of their determinations, it should have done so in these winged intermediates. The second of the three explanations just referred to requires that, at low temperature, the nature of the tibiae be determined earlier than that of the reproductive system. The correlation between wings and tibiae shows that the tibiae are determined quite late at low tempera-

ture, hence it is scarcely possible that the reproductive system is determined still later.

It appears practically certain, therefore, that the second possible explanation of my earlier experiments must be abandoned. The first and third, one of them consistent with Goldschmidt's theory of intersexes, the other not, remain as possibilities. It is hoped that the more complete study of intermediates which is in progress will throw light on the relative values of these two explanations.

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CHROMOSOME NUMBERS IN *PHLOX*

ANTECEDENT to a genetic investigation on the genus *Phlox*, chromosome counts were made of twenty-five varieties distributed among thirteen species. Kelly (1920) reported that the chromosome number in *Phlox drummondi* is fourteen for the cells of the root-tip. Kelly and Wahl, in a later report (1928) state that "many chromosome counts from pollen mother cells of different species of phlox by the Belling method have indicated that the number of chromosomes in the somatic cells of *P. drummondi, P. subulata, P. stolonifera, P. divaricata, P. pilosa, P. glaberrima, P. ovata, P. maculata* and *P. paniculata* is 14."

All the chromosome counts in the present investigation were made from bud material. The haploid counts were from pollen mother cells and the diploid counts were usually from the somatic tissue of the ovary. This latter tissue abounds with division figures in most species and it is not difficult to find stages in which the chromosomes may be counted. In most species division figures were equally numerous regardless of the time of day at which fixation occurred. The buds were fixed equally well in Bouin's fluid and in Allen's modification of Bouin's. Carnoy's fluid gave poor results. Following fixation the buds were dehydrated, cleared in xylol, and imbedded in paraffin. Sections cut at ten microns proved best for the study. The sections were stained in Haidenhain's iron-alum haematoxylin.

The chromosomes of all the species observed were relatively very large.

The following table gives the chromosome numbers and the source of the plants from which material was obtained for this study. The nomenclature is in most cases that of "Standardized Plant Names."

		N	*2N*
P. adsurgens	(Oregon Gardens)	–	20+
P. amoena Sims (*P. procumbens*)	(Dreer and Oregon Gardens)	6–8	14
P. arendsi varieties			
Charlotte	(Bobbink & Atkins)	–	14
Hanna	(" " ")	–	14
Hilda	(" " ")	7	14
P. argillacea Clute & Ferris	(Oregon Gardens)	7	14
P. diffusa	(Oregon Gardens)	–	28
P. divaricata L. (*P. canadensis* Sweet)		7	14
P. douglasii	(Oregon Gardens)	–	14
P. drummondi Hook. (several types including Star)		7	14
P. glaberrima suffruticosa var.			
Miss Lingard	(Valley View)	7	—
P. maculata L. var.			
Alpha	(Bobbink & Atkins)	7	14
P. ovata L. (*P. carolina* L.)	(Dreer)	7	14
P. paniculata L. varieties			
Eclaireur		–	14
Elizabeth Campbell		7	14
Mrs. Jenkins (Independence)		–	14
Mrs. Milly van Hoboken		–	14
Pantheon		7	14
Rheinlander		–	14
R. P. Struthers		7	14
Siebold		7	14

Sunset		–	14
Thor		–	14
	(various commercial seed houses)		
P. stolonifera Sims (*P. reptans* Michx.)	(Oregon Gardens)	–	14
P. subulata L. varieties			
rosea	(Valley View)	7	14
lilacina	(" ")	–	14

Only one plant of *Phlox adsurgens* was available. The chromosomes could not be counted in the pollen mother cells of any of the few buds which the plant produced in 1931. This species, unlike most of the others dealt with, does not show a profusion of division figures at all times of the day. In the several buds sectioned there were no good somatic figures. In several figures at least twenty chromosomes were counted. In these several chromosomes were so clumped as to make exact counting impossible. It appears probable that the chromosome number in this species may be twenty-one, in which case there is a possibility of its being a hybrid between a twenty-eight and a fourteen chromosome type, both of which are native to the same general region.

The pollen mother cells of *Phlox amoena* contain six, seven, and eight chromosomes. This appears to be due to irregular haplosis. There are from two to eight "early" chromosomes on practically every heterotypic spindle. At anaphase the chromosomes are scattered on the spindle from the equator to the poles. On some of these spindles seven chromosomes go to each pole. On others six chromosomes go to one pole and eight to the other. According to Bailey (1930) *Phlox amoena* is rarely cultivated—the plant grown under this name being *P. procumbens.* This is a supposed hybrid between *P. stolonifera* and *P. subulata.* The *P. amoena* plants which furnished the material used in this study are especially vigorous for *Phlox* plants, and spread very rapidly by runners. This—together with the irregularity of the chromosome behavior at haplosis—lends color to the supposition that the plants dealt with are a hybrid species.

In all the buds of *Phlox divaricata* var. *alba grandiflora* that have been examined the anthers are aborted. Apparently no good pollen is ever formed. Also the ovaries are aborted in over half the buds.

The ovaries of *Phlox stellaria* appear almost always to be aborted. One bud out of twenty-seven examined was found to have a developed ovary and in this the ovules were aborted.

Normal appearing pollen is formed. According to Bailey (1930) "the plant cultivated under this name is apparently a hybrid between *P. bifida* and *P. subulata.*" I was unable to count the chromosomes of *P. stellaria.*

During the summer of 1930 attempts were made to cross *Phlox paniculata* reciprocally with *Phlox drummondi.* Approximately sixty crosses were made each way. The crosses in which *P. drummondi* was used as the female parent resulted in three weak-appearing sets. These matured into capsules devoid of seed. No sets were secured from the reciprocal.

In the spring of 1931 attempts were made to cross *Phlox divaricata* reciprocally with *Phlox drummondi.* Forty crosses were made using the former as the female parent. Twenty-six sets resulted. From these twenty-three normal appearing seed were secured. Many of the capsules contained only one seed, or no seed, or seeds that were shriveled. No sets were obtained from thirty-four reciprocal crosses.

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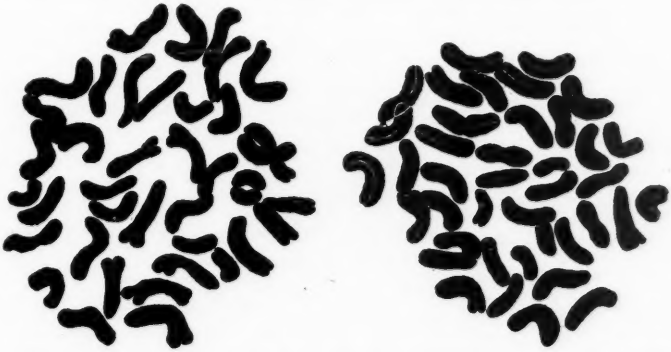
CHROMOSOME NUMBER IN SPECIES OF PEANUT, *ARACHIS*

PRIOR to a genetic investigation dealing with growth habit, testa color, and size and number of seeds per pod, the somatic chromosomes of six varieties of the commercial peanut, *Arachis hypogaea,* and a strain of the wild Brazilian species, *Arachis nambyquarae,* were counted. Though a study of the meiotic processes and haploid chromosomes is at the present time in progress, this note is merely intended to report the chromosome numbers encountered in root tip cells.

Commercial seed houses furnished the source of the *A. hypogaea* material. The *A. nambyquarae* seed was procured

through the kindness of the United States Department of Agriculture. The seeds of both species were germinated successfully within three days on moist filter-paper. The root tips were favorably fixed in Bouin's fluid and, by the usual method, imbedded in paraffin. Sections were cut 10 μ in thickness and stained with Haidenhain's iron-alum-haematoxylin.



The chromosomes of all the forms studied, though small and numerous, are well spaced and practically in a single plane at metaphase. The study and counting of them can be done, therefore, with no great difficulty and with little chance for error. The following table gives the diploid chromosome numbers and the specific source of the seed used.

A. nambyquarae (U. S. D. A.)	40
A. hypogaea	
California Long Red (Stumpp and Walter)	40
Improved Virginia (T. W. Wood and Sons)	40
Jumbo (Williams)	40
Spanish (T. W. Wood and Sons)	40
Valencia (T. W. Wood and Sons)	40
Virginia Bunch (T. W. Wood and Sons)	40

The accompanying figures (1. *A. nambyquarae*, 2. *A. hypogaea* var. Spanish), showing the size and general appearance of the chromosomes, were drawn at table level with the aid of an Abbe camera lucida, under a Leitz objective, 2 mm. N. A. 1.32, and a Zeiss compensating ocular 30×. The magnification is approximately 4500×.

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GRASS IN A TURTLE EGG

IN preparing embryos of turtles in late stages of development for embryological study at the Beaufort Laboratory, an egg was opened which had a rather well-developed stem of grass inside

FIG. 1. Ventral view showing grass *in situ*.

the shell. There was no apparent opening in the shell through which the grass might have entered and the writer is at loss to explain its presence. Fig. 1 is a photograph of the living embryo

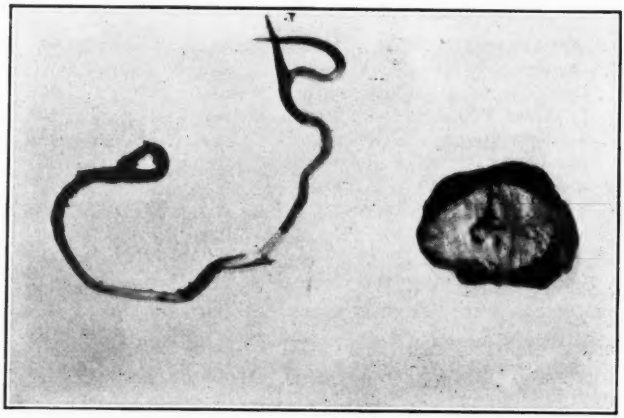

FIG. 2. A. Grass after growth. B. Ventral view of turtle, grass and membranes removed.

and grass in the position in which they occurred within the egg. It is to be noted that the embryo is about ready to hatch.

The grass was removed and placed in a Petri dish with moistened filter-paper. Under these conditions the grass grew for several days. Fig. 2 is a photograph of the grass and the turtle as they appeared when the grass was finally preserved; preservation being necessary on account of a fungus infection.

The grass has been identified by Dr. H. L. Blomquist, of Duke University, as *Spartina patens,* salt marsh grass.

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THE OLDEST MOTH

COCKERELL and LeVeque have just described (AM. NAT.: lxv, 354–355, Fig. 1) a moth under the name of *Chionaemopsis*

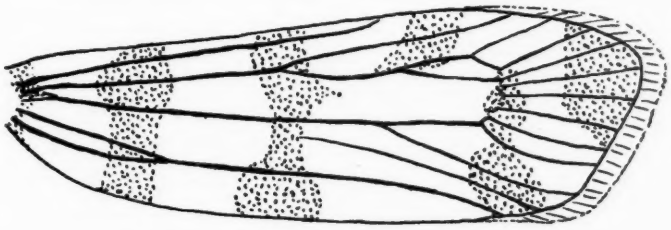

FIG. 1

quadrifasciatus. May I suggest that this is very possibly a species of the modern genus *Atteva* or its immediate ancestor.

On comparing the North American *Atteva aurea* (Fig. 1) with the fossil (Fig. 2) it is seen that the copper pattern cor-

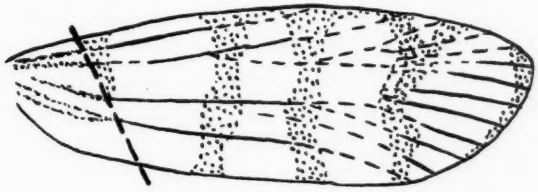

FIG. 2

responds with the dark pattern of the fossil, save that the two postmedial bands are complete in the latter, and that the vena-

tion so far as distinct is of the same type, but the broader wing suggests a transition to *Lactura* or *Urodus*. Cockerell and LeVeque's restoration of the venation can hardly be taken as final, as it would exclude the form from the Lepidoptera. The restoration dotted in on Fig. 2 fits with the traces visible on the half-tone figure, and would make a normal Yponomeutid.

If this form is really an *Atteva*, it is of great interest, as the genus is one of those now represented by well-marked subgroups in the tropics of the two hemispheres, and in addition appears to be a connecting link between the Yponomeutidae and the Pyralids.

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